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DOCUMENT-IDENTIFIER: US 6120751 A
TITLE: Charged lipids and uses for the same

Abstract Text (1):
The present invention is directed to charged lipids, compositions comprising charged lipids, and the use of these compositions in drug delivery, targeted drug delivery, therapeutic imaging and diagnostic imaging, as well as their use as contrast agents.

Brief Summary Text (2):
The present invention is directed to charged lipids, compositions comprising charged lipids, and the use of these compositions in drug delivery, targeted drug delivery, therapeutic imaging and diagnostic imaging, as well as their use as contrast agents.

Brief Summary Text (8):
The present invention is directed to, among other things, the development of new and improved drug and contrast media delivery systems that overcome the problems associated with the prior art.

Brief Summary Text (14):
In addition, the present invention describes novel contrast agents comprising a charged lipid, a counter ion, and a lipid covalently bonded to a polymer. The contrast agents may further comprise, for example, one or more of neutral lipids, charged lipids, gases, gaseous precursors, liquids, oils, diagnostic agents, targeting ligands and/or bioactive agents.

Detailed Description Text (19):
"Diagnostic agent" refers to any agent which may be used in connection with methods for imaging an internal region of a patient and/or diagnosing the presence or absence of a disease in a patient. Diagnostic agents include, for example, contrast agents for use in connection with ultrasound imaging, magnetic resonance imaging (MRI), nuclear magnetic resonance (NMR), computed tomography (CT), electron spin resonance (ESR), nuclear medical imaging, optical imaging, elastography, radiofrequency (RF) and microwave laser. Diagnostic agents may also include any other agents useful in facilitating diagnosis of a disease or other condition in a patient, whether or not imaging methodology is employed. As defined herein, a "diagnostic agent" is a type of bioactive agent.

Detailed Description Text (100):
In the compositions of the present invention, the lipids covalently bonded to polymers include, for example, lipids covalently bonded to hydrophilic polymers. Suitable hydrophilic polymers for covalent bonding to lipids include, for example, polyalkyleneoxides such as, for example, polyethylene glycol (PEG) and polypropylene glycol (PPG), polyvinyl-pyrrolidones, polyvinylalkylethers, such as a polyvinylmethyl ether, polyacrylamides, such as, for example, polymethacrylamides, polydimethylacrylamides and polyhydroxy-propylmethacrylamides, polyhydroxyalkyl(meth)acrylates, such as polyhydroxyethyl acrylates, polyhydroxypropyl methacrylates, polyalkyloxazolines, such as polymethyloxazolines and polyethyloxazolines, polyhydroxyalkyloxazolines, such as polyhydroxyethyloxazolines, polyhyhydroxypropyloxazolines, polyvinyl alcohols, polyphosphazenes, poly(hydroxy-alkylcarboxylic acids), polyoxazolidines,

polyaspartamide, and polymers of sialic acid (polysialics). Preferably, the hydrophilic polymers are polyethylene glycol, polyvinyl pyrrolidone, polyvinyl alcohol, polypropylene glycol, a polyvinylalkylether, a polyacrylamide, a polyalkyloxazoline, a polyhydroxyalkyloxazoline, a polyphosphazene, a polyoxazolidine, a polyaspartamide, a polymer of sialic acid, a polyhydroxyalkyl(meth)acrylate or a poly(hydroxyalkylcarboyxlic acid). More preferably, the hydrophilic polymers are PEG, PPG, polyvinylalcohol, polyvinylpyrrolidone and copolymers thereof, with PEG and PPG polymers being more preferred and PEG polymers being even more prefered. The polyethylene glycol may be, for example, PEG 2000, PEG 5000 or PEG 8000, which have weight average molecular weights of 2000, 5000 and 8000 daltons, respectively. Preferably, the polyethylene glycol has a molecular weight of about 500 to about 20,000, more preferably from about 1,000 to about 10,000. Other suitable polymers, hydrophilic and otherwise, will be apparent to one skilled in the art based on the present disclosure. Polymers which may be attached to the lipid via alkylation or acylation reactions onto the surface of the liposome are particularly useful for improving the stability and size of the distribution of the liposomes. Exemplary lipids which are covalently bonded to hydrophilic polymers include, for example, dipalmitoylphosphatidylethanolamine-PEG, dioleoylphosphatidylethanolamine-PEG and distearylphosphatidylethanolamine-PEG, more preferably dipalmitoylphosphatidylethanolamine-PEG.

Detailed Description Text (101):
In addition to the anionic and cationic lipids described above, other suitable lipids which may be used in the present invention include, for example, fatty acids, lysolipids, fluorinated lipids, phosphocholines, such as those associated with platelet activation factors (PAF) (Avanti Polar Lipids, Alabaster, AL), including 1-alkyl-2-acetoyl-sn-glycero 3-phosphocholines, and 1-alkyl-2-hydroxy-sn-glycero 3-phosphocholines, which target blood clots; phosphatidylcholine with both saturated and unsaturated lipids, including dioleoylphosphatidylcholine; dimyristoylphosphatidylcholine (DMPC); dipentadecanoylphosphatidylcholine; dilauroylphosphatidylcholine; dipalmitoylphosphatidylcholine (DPPC); distearoylphosphatidylcholine (DSPC); and diarachidonylphosphatidylcholine (DAPC); phosphatidylethanolamines, such as dioleoylphosphatidylethanolamine, dimyristoylphosphatidylethanolamine (DMPE), dipalmitoylphosphatidylethanolamine (DPPE) and distearoylphosphatidylethanolamine (DSPE); phosphatidylserine; phosphatidylglycerols, including distearoylphosphatidylglycerol (DSPG); phosphatidylinositol; sphingolipids such as sphingomyelin; glycolipids such as ganglioside GM1 and GM2; glucolipids; sulfatides; glycosphingolipids; phosphatidic acids, such as dipalmitoylphosphatidic acid (DPPA) and distearoylphosphatidic acid (DSPA); palmitic acid; stearic acid; arachidonic acid; oleic acid; linolenic acid; linoleic acid; myristic acid; synthetic lipids described in U.S. Pat. No. 5,312,617, the disclosure of which is hereby incorporated by reference herein in its entirety; lipids bearing polymers, such as-chitin, hyaluronic acid, polyvinylpyrrolidone or polyethylene glycol (PEG), also referred to herein as "pegylated lipids" with preferred lipid bearing polymers including DPPE-PEG (DPPE-PEG), which refers to the lipid DPPE having a PEG polymer attached thereto, including, for example, DPPE-PEG5000, which refers to DPPE having attached thereto a PEG polymer having a mean average molecular weight of about 5000; lipids bearing sulfonated mono-, di-, oligo- or polysaccharides; cholesterol, cholesterol sulfate and cholesterol hemisuccinate; tocopherol hemisuccinate; lipids with ether and ester-linked fatty acids; polymerized lipids (a wide variety of which are known in the art); diacetyl phosphate; dicetyl phosphate; stearylamine; cardiolipin; phospholipids with short chain fatty acids of about 6 to about 8 carbons in length; synthetic phospholipids with asymmetric acyl chains, such as, for example, one acyl chain of about 6 carbons and another acyl chain of about 12 carbons; ceramides; non-ionic liposomes including niosomes such as polyoxyalkylene (e.g., polyoxyethylene) fatty acid esters, polyoxyalkylene (e.g., polyoxyethylene) fatty alcohols, polyoxyalkylene (e.g., polyoxyethylene) fatty alcohol ethers, polyoxyalkylene (e.g., polyoxyethylene) sorbitan fatty acid esters (such as the class of compounds referred to as TWEEN.RTM., including, for example, TWEEN.RTM. 20, TWEEN.RTM. 40 and TWEEN.RTM. 80, commercially available from ICI Americas, Inc., Wilmington, Del.), glycerol polyethylene glycol oxystearate, glycerol polyethylene glycol ricinoleate, alkyloxylated (e.g., ethoxylated) soybean sterols, alkyloxylated (e.g., ethoxylated) castor oil, polyoxyethylene-polyoxypropylene polymers, and polyoxyalkylene (e.g.,

polyoxyethylene) fatty acid stearates; sterol aliphatic acid esters including cholesterol sulfate, cholesterol butyrate, cholesterol isobutyrate, cholesterol palmitate, cholesterol stearate, lanosterol acetate, ergosterol palmitate, and phytosterol n-butyrate; sterol esters of sugar acids including cholesterol glucuronide, lanosterol glucuronide, 7-dehydrocholesterol glucuronide, ergosterol glucuronide, cholesterol gluconate, lanosterol gluconate, and ergosterol gluconate; esters of sugar acids and alcohols including lauryl glucuronide, stearoyl glucuronide, myristoyl glucuronide, lauryl gluconate, myristoyl gluconate, and stearoyl gluconate; esters of sugars and aliphatic acids including sucrose laurate, fructose laurate, sucrose palmitate, sucrose stearate, glucuronic acid, gluconic acid and polyuronic acid; saponins including sarsasapogenin, smilagenin, hederagenin, oleanolic acid, and

Detailed Description Text (147):
Polymers useful to stabilize the vesicles of the present invention may be of natural, semi-synthetic (modified natural) or synthetic origin. Suitable natural polymers include naturally occurring polysaccharides, such as, for example, arabinans, fructans, fucans, galactans, galacturonans, glucans, mannans, xylans (such as, for example, inulin), levan, fucoidan, carrageenan, galatocarolose, pectic acid, pectins, including amylose, pullulan, glycogen, amylopectin, cellulose, dextran, dextrin, dextrose, glucose, polyglucose, polydextrose, pustulan, chitin, agarose, keratin, chondroitin, dermatan, hyaluronic acid, alginic acid, xanthin gum, starch and various other natural homopolyner or heteropolymers, such as those containing one or more of the following aldoses, ketoses, acids or amines: erythrose, threose, ribose, arabinose, xylose, lyxose, allose, altrose, glucose, dextrose, mannose, gulose, idose, galactose, talose, erythrulose, ribulose, xylulose, psicose, fructose, sorbose, tagatose, mannitol, sorbitol, lactose, sucrose, trehalose, maltose, cellobiose, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, glucuronic acid, gluconic acid, glucaric acid, galacturonic acid, mannuronic acid, glucosamine, galactosamine, and neuraminic acid, and naturally occurring derivatives thereof Accordingly, suitable polymers include, for example, proteins, such as albumin, polyalginates, and polylactide-coglycolide polymers. Exemplary semi-synthetic polymers include carboxymethylcellulose, hydroxymethylcellulose, hydroxypropylmethylcellulose, methylcellulose, and methoxycellulose. Exemplary synthetic polymers include polyphosphazenes, hydroxyapatites, fluoroapatite polymers, polyethylenes (such as, for example, polyethylene glycol (including for example, the class of compounds referred to as Pluronics.RTM., commercially available from BASF, Parsippany, N.J.), polyoxyethylene, and polyethylene terephthlate), polypropylenes (such as, for example, polypropylene glycol), polyurethanes (such as, for example, polyvinyl alcohol (.PVA), polyvinyl chloride and polyvinylpyrrolidone), polyamides including nylon, polystyrene, polylactic acids, fluorinated hydrocarbon polymers, fluorinated carbon polymers (such as, for example, polytetrafluoroethylene), acrylate, methacrylate, and polymethylmethacrylate, and derivatives thereof. Methods for the preparation of vesicles which employ polymers to stabilize vesicle compositions will be readily apparent to one skilled in the art, in view of the present disclosure, when coupled with information known in the art, such as that described and referred to in Unger, U.S. Pat. No. 5,205,290, the disclosure of which is hereby incorporated by reference herein in its entirety.

Detailed Description Text (149):
their phosphorylated and sulfonal:ed derivatives; polyethers, preferably with molecular weight ranges between 400 and 100,000; and di- and trihydroxy alkanes and their polymers, preferably with molecular weight ranges between 200 and 50,000; (ii) emulsifying and/or solubilizing agents including, for example, acacia, cholesterol, diethanolamine, glyceryl monostearate, lanolin alcohols, lecithin, mono- and di-glycerides, mono-ethanolamine, oleic acid, oleyl alcohol, poloxamer, for example, poloxamer 188, poloxamer 184, and poloxamer 181, Pluronics.RTM. (BASF, Parsippany, N.J.), polyoxyethylene 50 stearate, polyoxyl 35 castor oil, polyoxl 10 oleyl ether, polyoxyl 20 cetostearyl ether, polyoxyl 40 stearate, polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, propylene glycol diacetate, propylene glycol monostearate, sodium lauryl sulfate, sodium stearate, sorbitan monolaurate, sorbitan monooleate, sorbitan monopalmitate, sorbitan monostearate, stearic acid, trolamine, and emulsifying wax; (iii) suspending and/or viscosity-increasing agents, including,

for example, acacia, agar, alginic acid, aluminum monostearate, bentonite, magma, carbomer 934P, carboxymethylcellulose, calcium and sodium and sodium 12, carrageenan, cellulose, dextran, gelatin, guar gum, locust bean gum, veegum, hydroxyethyl cellulose, hydroxypropyl methylcellulose, magnesium-aluminum-silicate, Zeolites.RTM., methylcellulose, pectin, polyethylene oxide, povidone, propylene glycol alginate, silicon dioxide, sodium alginate, tragacanth, xanthin gum, .alpha.-d-gluconolactone, glycerol and mannitol; (iv) synthetic suspending agents, such as polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), polyvinylalcohol (PVA), polypropylene glycol (PPG), and polysorbate; and (v) tonicity raising agents which stabilize and add tonicity, including, for example, sorbitol, mannitol, trehalose, sucrose, propylene glycol and glycerol.

Detailed Description Text (151):
The present stabilizing materials or compositions preferably comprise a gas, such as an inert gas. The gas provides the stabilizing materials or compositions with enhanced reflectivity, particularly in connection with stabilizing materials or compositions in which the gas is entrapped within the stabilizing materials or compositions. This may increase their effectiveness as drug delivery vehicles or contrast agents.

Detailed Description Text (172):
A wide variety of bioactive agents may be entrapped in the compositions of the present invention. Suitable bioactive agents include, for example, contrast agents, genetic materials, chemotherapeutics, peptides and nucleic acids. The compositions of the present invention may also be used for stabilizing gas bodies for use in ultrasound and drug delivery. Preferably, the bioactive agent is genetic material, which includes, for example, nucleic acids, RNA and DNA, of either natural or synthetic origin, including recombinant RNA and DNA and antisense RNA and DNA, hammerhead RNA, ribozymes, hammerhead ribozymes, antigene nucleic acids, both single and double stranded RNA and DNA and analogs thereof, ribooligonucleotides, deoxyribooligonucleotides, antisense ribooligonucleotides, and antisense deoxyribooligonucleotides.

Detailed Description Text (222):
The targeting ligands may be linked or attached to the compositions of the present invention via a linking group. A variety of linking groups are available and would be apparent to one skilled in the art in view of the present disclosure. Preferably, the linking group comprises a hydrophilic polymer. Suitable hydrophilic polymers include, for example, polyalkyleneoxides such as, for example, polyethylene glycol (PEG) and polypropylene glycol (PPG), polyvinylpyrrolidones, polyvinylmethylethers, polyacrylamides, such as, for example, polymethacrylamides, polydimethylacrylamides and polyhydroxypropylmethacrylamides, polyhydroxyethyl acrylates, polyhydroxypropyl methacrylates, polyalkyloxazolines, such as polymethyloxazolines and polyethyloxazolines, polyhydroxyalkyloxazolines, such as polyhydroxyethyloxazolines, polyhyhydroxypropyloxazolines, polyvinyl alcohols, polyphosphazenes, poly(hydroxyalkylcarboxylic acids), polyoxazolidines, polyaspartamide, and polymers of sialic acid (polysialics). The hydrophilic polymers are preferably selected from the group consisting of PEG, PPG, polyvinylalcohol and polyvinylpyrrolidone and copolymers thereof, with PEG and PPG polymers being more preferred and PEG polymers being even more prefered. Thus, in embodiments involving lipid compositions which comprise lipids bearing polymers including, for example, DPPE-PEG, the targeting ligand may be linked directly to the polymer which is attached to the lipid to provide, for example, a conjugate of DPPE-PEG-TL, where TL is a targeting ligand. Thus, using the example DPPE-PEG, such as, for example, DPPE-PEG5000, the aforementioned conjugate may be represented as DPPE-PEG5000-TL. The hydrophilic polymer used as a linking group is preferably a bifunctional polymer, for example, bifunctional PEG, such as diamino-PEG. In this case, one end of the PEG group is linked, for example, to a lipid compound, and is bound at the free end to the targeting ligand via an amide linkage. A hydrophilic polymer, for example, PEG, substituted with a terminal carboxylate group on one end and a terminal amino group on the other end, may also be used. These latter bifunctional hydrophilic polymer may be preferred since they possess various similarities to amino acids.

Detailed Description Text (242):
In the above compounds, P is a hydrophilic polymer. Preferably, P is a hydrophilic

polymer selected from the group consisting of polyalkyleneoxides, polyvinyl alcohol, polyvinylpyrrolidones, polyacrylamides, polymethacrylamides, polyphosphazenes, phosphazene, poly(hydroxyalkylcarboxylic acids) and polyoxazolidines. More preferably, P is a polyalkyleneoxide polymer, with polyethylene glycol and polypropylene glycol being even more preferred and polyethylene glycol being particularly preferred.

Detailed Description Text (252):
In the above formula, Z is a hydrophilic polymer. Preferably, Z is selected from the group consisting of polyalkyleneoxides, polyvinyl alcohol, polyvinylpyrrolidones, polyacrylamides, polymethacrylamides, polyphosphazenes, poly(hydroxyalkylcarboxylic acids) and polyoxazolidines. More preferably, Z comprises a polyalkyleneoxide. Even more preferably, Z is a polyalkyleneoxide selected from the group consisting of polyethylene glycol and polypropylene glycol, with polyethylene glycol being still more preferred. In certain other preferred embodiments, Z is a hydrophilic polymer other than polyalkylene-oxides, including polyethylene glycol and polypropylene glycol. The molecular weight of Z may vary, depending, for example, on the particular end-use of the compounds. Preferably, Z is a polymer having a molecular weight which ranges from about 100 to about 10,000, and all combinations and subcombinations of ranges therein. More preferably, Z is a polymer having a molecular weight of from about 1,000 to about 5,000. Also preferred are polymers which exhibit polydispersities ranging from greater than about 1 to about 3, and all combinations and subcombinations of ranges therein. More preferably, Z is a polymer having a polydispersity of from greater than about 1 to about 2, with polydispersities of from greater than about 1 to about 1.5 being even more preferred, and polydispersities of from greater than about 1 to about 1.2 being still more preferred.

Detailed Description Text (308):
Conventional, aqueous-filled liposomes of the prior art are routinely formed at a temperature above the phase transition temperature of the lipids used to make them, since they are more flexible and thus useful in biological systems in the liquid crystalline state. See, Szoka and Papahadjopoulos, Proc. Natl. Acad. Sci. (1978) 75, 4194-4198. In contrast, the vesicles made according to embodiments described herein are gaseous precursor filled, which imparts greater flexibility, since gaseous precursors after gas formation are more compressible and compliant than an aqueous solution.

Detailed Description Text (341):
Ultrasound can be used for both diagnostic and therapeutic purposes. In diagnostic ultrasound, ultrasound waves or a train of pulses of ultrasound may be applied with a transducer. The ultrasound is generally pulsed rather than continuous, although it may be continuous, if desired. Thus, diagnostic ultrasound generally involves the application of a pulse of echoes, after which, during a listening period, the ultrasound transducer receives reflected signals. Harmonics, ultraharmonics or subharmonics may be used. The second harmonic mode may be beneficially employed, in which the 2x frequency is received, where x is the incidental frequency. This may serve to decrease the signal from the background material and enhance the signal from the transducer using the targeted contrast media of the present invention which may be targeted to the desired site, for example, blood clots. Other harmonic signals, such as odd harmonics signals, for example, 3x or 5x, would be similarly received using this method. Subharmonic signals, for example, x/2 and x/3, may also be received and processed so as to form an image.

Detailed Description Text (365):
As discussed above, the compositions and stabilizing materials of the present invention may be used in connection with diagnostic imaging, therapeutic imaging and drug delivery, including, for example, ultrasound (US), magnetic resonance imaging (MRI), nuclear magnetic resonance (NMR), computed tomography (CT), electron spin resonance (ESR), nuclear medical imaging, optical imaging, elastography, radiofrequency (RF) and microwave laser. The compositions and stabilizing materials of the present invention may be used in combination with various contrast agents, including conventional contrast agents, which may serve to increase their effectiveness as contrast agents for diagnostic and therapeutic imaging.

Detailed Description Text (366):
Examples of suitable contrast agents for use with MRI in combination with the present stabilizing materials include, for example, stable free radicals, such as, stable nitroxides, as well as compounds comprising transition, lanthanide and actinide elements, which may, if desired, be in the form of a salt or may be covalently or non-covalently bound to complexing agents, including lipophilic derivatives thereof, or to proteinaceous macromolecules. Preferable transition, lanthanide and actinide elements include, for example, Gd(III), Mn(II), Cu(II), Cr(III), Fe(II), Fe(III), Co(II), Er(II), Ni(II), Eu(III) and Dy(III). More preferably, the elements may be Gd(III), Mn(II), Cu(II), Fe(II), Fe(III), Eu(III) and Dy(III), most preferably Mn(II) and Gd(III). The foregoing elements may be in the form of a salt, including inorganic salts, such as a manganese salt, for example, manganese chloride, manganese carbonate, manganese acetate, and organic salts, such as manganese gluconate and manganese hydroxyl- apatite. Other exemplary salts include salts of iron, such as iron sulfides, and ferric salts, such as ferric chloride.

Detailed Description Text (368):
Nitroxides are paramagnetic contrast agents which increase both T1 and T2 relaxation rates on MRI by virtue of the presence of an unpaired electron in the nitroxide molecule. As known to one of ordinary skill in the art, the paramagnetic effectiveness of a given compound as an MRI contrast agent may be related, at least in part, to the number of unpaired electrons in the paramagnetic nucleus or molecule, and specifically, to the square of the number of unpaired electrons. For example, gadolinium has seven unpaired electrons whereas a nitroxide molecule has one unpaired electron. Thus, gadolinium is generally a much stronger MRI contrast agent than a nitroxide. However, effective correlation time, another important parameter for assessing the effectiveness of contrast agents, confers potential increased relaxivity to the nitroxides. When the tumbling rate is slowed, for example, by attaching the paramagnetic contrast agent to a large molecule, it will tumble more slowly and thereby more effectively transfer energy to hasten relaxation of the water protons. In gadolinium, however, the electron spin relaxation time is rapid and will limit the extent to which slow rotational correlation times can increase relaxivity. For nitroxides, however, the electron spin correlation times are more favorable and tremendous increases in relaxivity may be attained by slowing the rotational correlation time of these molecules. The gas filled vesicles of the present invention are ideal for attaining the goals of slowed rotational correlation times and resultant improvement in relaxivity. Although not intending to be bound by any particular theory of operation, it is contemplated that since the nitroxides may be designed to coat the perimeters of the vesicles, for example, by making alkyl derivatives thereof, the resulting correlation times can be optimized. Moreover, the resulting contrast medium of the present invention may be viewed as a magnetic sphere, a geometric configuration which maximizes relaxivity.

Detailed Description Text (369):
Exemplary superparamagnetic contrast agents suitable for use with MRI in the compositions of the present invention include metal oxides and sulfides which experience a magnetic domain, ferro- or ferrimagnetic compounds, such as pure iron, magnetic iron oxide, such as magnetite, .gamma.-Fe.sub.2 O.sub.3, Fe.sub.3 O.sub.4, manganese ferrite, cobalt ferrite and nickel ferrite. Paramagnetic gases can also be employed in the present compositions, such as oxygen 17 gas (.sup.17 O.sub.2). In addition, hyperpolarized xenon, neon, or helium gas may also be employed. Magnetic resonance (MR) whole body imaging may then be employed to rapidly screen the body, for example, for thrombosis, and ultrasound may be applied, if desired, to aid in thrombolysis.

Detailed Description Text (370):
The contrast agents, such as the paramagnetic and superparamagnetic contrast agents described above, may be employed as a component within the compositionss and/or stabilizing materials. With respect to vesicles, the contrast agents may be entrapped within the internal void thereof, administered as a solution with the vesicles;, incorporated with any additional stabilizing materials, or coated onto the surface or membrane of the vesicle. Mixtures of any one or more of the paramagnetic agents and/or superparamagnetic agents in the present compositions may be used. The paramagnetic and superparamagnetic agents may also be coadministered

separately, if desired.

Detailed Description Text (372):
The stabilizing materials and/or vesicles of the present invention, and especially the vesicles, may serve not only as effective carriers of the superparamagnetic agents described above, but also may improve the effect of the susceptibility contrast agents. Superparamagnetic contrast agents include metal oxides, particularly iron oxides but including manganese oxides, and as iron oxides, containing varying amounts of manganese, cobalt and nickel which experience a magnetic domain. These agents are nano or microparticles and have very high bulk susceptibilities and transverse relaxation rates. The larger particles, for example, particles having diameters of about 100 nm, have much higher R2 relaxivities as compared to R1 relaxivities. The smaller particles, for example, particles having diameters of about 10 to about 15 nm, have somewhat lower R2 relaxivities, but much more balanced R1 and R2 values. Much smaller particles, for example, monocrystalline iron oxide particles having diameters of about 3 to about 5 nm, have lower R2 relaxivities, but probably the most balanced R1 and R2 relaxation rates. Ferritin can also be formulated to encapsulate a core of very high relaxation rate superparamagnetic iron. It has been discovered that the lipid and/or vesicle compositions, especially vesicle compositions, including gas filled vesicles, can increase the efficacy and safety of these conventional iron oxide based MRI contrast agents.

Detailed Description Text (374):
Without being bound to any particular theory or theories of operation, it is believed that the vesicles of the present invention increase the efficacy of the superparamagnetic contrast agents by several mechanisms. First, it is believed that the vesicles function to increase the apparent magnetic concentration of the iron oxide particles. Also, it is believed that the vesicles increase the apparent rotational correlation time of the MRI contrast agents, including paramagnetic and superparamagnetic agents, so that relaxation rates are increased. In addition, the vesicles appear to increase the apparent magnetic domain of the contrast medium according to the manner described hereinafter.

Detailed Description Text (376):
differing susceptibility from the suspending medium, including, for example, the aqueous suspension of the contrast medium or blood or other body fluids, for example, in the case of intravascular injection or injection into other body locations. In the case of ferrites or iron oxide particles, it should be noted that the contrast provided by these agents is dependent on particle size. This phenomenon is very common and is often referred to as the "secular" relaxation of the water molecules. Described in more physical terms, this relaxation mechanism is dependent upon the effective size of the molecular complex in which a paramagnetic atom, or paramagnetic molecule, or molecules, may reside. One physical explanation may be described in the following Solomon-Bloembergen equations which define the paramagnetic contributions as a function of the T.sub.1 and T.sub.2 relaxation times of a spin 1/2 nucleus with gyromagnetic ratio g perturbed by a paramagnetic ion: 1/T.sub.1 M=(2/15) S(S+1).gamma..sup.2 g.sup.2 .beta..sup.2 /r.sup.6 [3.tau..sub.c /(1+.omega..sub.I.sup.2 .tau..sub.c.sup.2)+7.tau..sub.c /(1+.omega..sub.s.sup.2 .tau..sub.c.sup.2)]+(2/3) S(S+1) A.sup.2 /h.sup.2 [.tau..sub.c /(1+.omega..sub.s 2.tau..sub.c.sup.2)] and 1/T.sub.2 M=(1/15) S(S+1) .gamma..sup.2 g.sup.2 .beta..sup.2 /r.sup.6 [4.tau..sub.c +3.tau.c/(1+.omega..sub.I.sup.2 .tau..sub.c.sup.2)+13.tau..sub.c /(1+w.sub.s.sup.2 .tau..sub.c.sup.2)]+(1/3) S(S+1)A.sup.2 /h.sup.2 [.tau..sub.c /(1+.omega..sub.s 2.tau..sub.c.sup.2)], where S is the electron spin quantum number; g is the electronic g factor; .beta. is the Bohr magneton; .omega..sub.I and .omega..sub.s (657 w.sub.I) is the Larmor angular precession frequencies for the nuclear spins and electron spins; r is the ion-nucleus distance; A is the hyperfine coupling constant; .tau..sub.c and .tau..sub.e are the correlation times for the dipolar and scalar interactions, respectively; and h is Planck's constant.

Detailed Description Text (377):
A few large particles may have a much greater effect than a larger number of much smaller particles, primarily due to a larger correlation time. If one were to make the iron oxide particles very large however, increased toxicity may result, and the

lungs may be embolized or the complement cascade system may be activated. Furthermore, it is believed that the total size of the particle is not as important as the diameter of the particle at its edge or outer surface. The domain of magnetization or susceptibility effect falls off exponentially from the surface of the particle. Generally speaking, in the case of dipolar (through space) relaxation mechanisms, this exponential fall off exhibits an r.sup.6 dependence for a paramagnetic dipole-dipole interaction. Interpreted literally, a water molecule that is 4 angstroms away from a paramagnetic surface will be influenced 64 times less than a water molecule that is 2 angstroms away from the same paramagnetic surface. The ideal situation in terms of maximizing the contrast effect would be to make the iron oxide particles hollow, flexible and as large as possible. It has not been possible to achieve this heretofore and it is believed that the benefits have been unrecognized heretofore also. By coating the inner or outer surfaces of the vesicles with the contrast agents, even though the individual contrast agents, for example, iron oxide nanoparticles or paramagnetic ions, are relatively small structures, the effectiveness of the contrast agents may be greatly enhanced. In so doing, the contrast agents may function as an effectively much larger sphere wherein the effective domain of magnetization is determined by the diameter of the vesicle and is maximal at the surface of the vesicle. These agents afford the advantage of flexibility, namely, compliance. While rigid vesicles might lodge in the lungs or other organs and cause toxic reactions, these flexible vesicles slide through the capillaries much more easily.

Detailed Description Text (378):
In contrast to the flexible vesicles described above, it may be desirable, in certain circumstances, to formulate vesicles from substantially impermeable polymeric materials including, for example, polymethyl methacrylate. This would generally result in the formation of vesicles which may be substantially impermeable and relatively inelastic and brittle. In embodiments involving diagnostic imaging, for example, ultrasound, contrast media which comprise such brittle vesicles would generally not provide the desirable reflectivity that the flexible vesicles may provide. However, by increasing the power output on ultrasound, the brittle microspheres can be made to rupture, thereby causing acoustic emissions which can be detected by an ultrasound transducer.

Other Reference Publication (8):
Cheng, et al., "The Production and Evaluation of Contrast-Carrying Liposomes Made with an Automatic High Pressure System", Investigative Radiology, vol. 22, pp. 47-55 (1987).

Other Reference Publication (13):
Mattrey et al., "Perfluorochemicals as US Contrast Agents for Tumor-Imaging and Hepatosplenography: Preliminary Clinical Results", Radiology, vol. 163, pp. 339-343 (1987).

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Mattrey et al., "Perfluoroctylbromide: A Liver/Spleen-Specific and Tumor Imaging Ultlrasound Contrast Material", Radiology, vol. 145, pp. 759-762 (1982).

Other Reference Publication (15):
Keller et al., "Successful Left Ventricular Opacification Following Peripheral Venous Injection of Sonicated Contrast Agent: An Experimental Evaluation", LV Contrast Echocardiography, vol. 114, No. 3, pp. 570-575 (1987).

Other Reference Publication (16):
Feinstein et al., "Two-Dimensional Contrast Echocardiography, I: In Vitro Development and Quantitative Analysis of Echo Contrast Agents", JACC, vol. 3, No. 1, pp. 14-20 (1984).

Other Reference Publication (17):
Ten Cate et al., "Two-Dimensional Contrast Echocardiography, II: Transpulmonary Studies", JACC, vol. 3, No. 1, pp. 21-27 (1984).

Other Reference Publication (28):
Violante et al., "Particulate Suspensions as Ultrasonic Contrast Agents for Liver

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CLAIMS:

1. A contrast agent comprising a cochleate which comprises a charged lipid, a counter ion, a lipid covalently bonded to a polymer, and a gas, a gaseous precursor or a gas and a gaseous precursor, wherein the gas and gaseous precursor comprise fluorinated compounds.

2. The contrast agent of claim 1, wherein the charged lipid is an anionic lipid and the counter ion is a cationic counter ion.

3. The contrast agent of claim 2, wherein the anionic lipid is selected from the group consisting of a phosphatidic acid, a phosphatidyl glycerol, a phosphatidyl glycerol fatty acid ester, a phosphatidyl ethanolamine anandamide, a phosphatidyl ethanolamine methanandamide, a phosphatidyl serine, a phosphatidyl inositol, a phosphatidyl inositol fatty acid ester, a cardiolipin, a phosphatidyl ethylene glycol, an acidic lysolipid, a sulfolipid, a sulfatide, a saturated free fatty acid, an unsaturated free fatty acid, a palmitic acid, a stearic acid, an arachidonic acid, an oleic acid, a linolenic acid, a linoleic acid, and a myristic acid.

4. The contrast agent of claim 2, wherein the cationic counter ion is selected from the group consisting of Be.sup.2+, Mg.sup.2+, Ca.sup.2+, Sr2+, Ba.sup.2+, Al.sup.3+, Ga.sup.3+, Ge.sup.3+, Sn.sup.4+, Pb.sup.2+, Pb.sup.4+, Ti.sup.3+, Ti.sup.4+, V.sup.2+, V.sup.3+, Cr.sup.2+, Cr.sup.3+, Mn.sup.2+, Mn.sup.3+, Fe.sup.2+, Fe.sup.3+, Co.sup.2+, Co.sup.3+, Ni.sup.2+, Ni.sup.3+, Cu.sup.2+, Zn.sup.2+, Zr.sup.4+, Nb.sup.3+, Mo.sup.2+, Mo.sup.3+, Cd.sup.2+, In.sup.3+, W.sup.2+, W.sup.4+, Os.sup.2+, Os.sup.3+, Os.sup.4+, Ir.sup.2+, Ir.sup.3+, Ir.sup.4+, Hg.sup.2+, Bi.sup.3+, La.sup.3+, and Gd.sup.3+.

5. The contrast agent of claim 4, wherein the cationic counter ion is selected from the group consisting of Ca.sup.2+, Mg.sup.2+, Zn.sup.2+, Mn.sup.2+ and Gd.sup.3+.

6. The contrast agent of claim 5, wherein the cationic counter ion is Ca.sup.2+.

7. The contrast agent of claim 1, wherein, in the lipid covalently bonded to the polymer, the polymer is selected from the group consisting of polyethylene glycol, polyvinyl pyrrolidone, polyvinyl alcohol, polypropylene glycol, a polyvinylalkylether, a polyacrylamide, a polyalkyloxazoline, a polyhydroxyalkyloxazoline, a polyphosphazene, a polyoxazolidine, a polyaspartamide, a polymer of sialic acid, a polyhydroxyalkyl(meth)acrylate and a poly(hydroxyalkylcarboyxlic acid).

8. The contrast agent of claim 7, wherein, in the lipid covalently bonded to the polymer, the polymer is polyethylene glycol.

9. The contrast agent of claim 8, wherein the polyethylene glycol has a molecular weight of from about 1,000 to about 10,000.

10. The contrast agent of claim 1, wherein the lipid covalently bonded to the polymer is selected from the group consisting of

11. The contrast agent of claim 2, wherein the anionic lipid is dipalmitoyl-phosphatidic acid, the cationic counter ion is Ca.sup.2+ and the lipid covalently bonded to the polymer is dipalmitoylphosphatidylethanolamine-polyethylene glycol.

12. The contrast agent of claim 1, further comprising at least one lipid having a neutral charge.

13. The contrast agent of claim 1, further comprising a targeting ligand.

14. The contrast agent of claim 13, wherein the targeting ligand is selected from the group consisting of peptides, proteins and saccharides.

15. The contrast agent of claim 1, wherein the fluorinated compound is selected from the group consisting of a perflurocarbon, sulfur hexafluoride and a perfluoroether.

16. The contrast agent of claim 15, wherein the fluorinated compound is a perfluorocarbon selected from the group consisting of perfluoromethane, perfluoroethane, perfluoropropane, perfluorocyclopropane, perfluorobutane,

17. The contrast agent of claim 15, wherein the fluorinated compound is a perfluoroether selected from the group consisting of perfluorotetrahydropyran, perfluoromethyltetrahydrofuran, perfluorobutylmethyl ether, perfluoropropylethyl ether, perfluorocyclobutylmethyl ether, perfluorocyclopropylethyl ether, perfluoropropylmethyl ether, perfluorodiethyl ether, perfluorocyclopropylmethyl ether, perfluoromethylethyl ether and perfluorodimethyl ether.

18. The contrast agent of claim 1, further comprising a fluorinated liquid.

19. The contrast agent of claim 18, wherein the fluorinated liquid is selected from the group consisting of perfluorohexane, perfluoroheptane, perfluorooctane, perfluorononane, perfluorodecane, perfluorododecane, perfluorocyclohexane, perfluorodecalin, perfluorododecalin, perfluorooctyliodide, perfluorooctylbromide, perfluorotripropylamine, perfluorotributylamine, perfluorobutylethyl ether, bis(perfluoroisopropyl) ether and bis(perfluoropropyl) ether.

20. A contrast agent of claim 1 wherein said cochleate is in the form of a spiral or tubule.

L7: Entry 298 of 424 File: USPT

DOCUMENT-IDENTIFIER: US 6028066 A
TITLE: Prodrugs comprising fluorinated amphiphiles

Brief Summary Text (25):
"Vesicle" refers to an entity which is generally characterized by the presence of one or more walls or membranes which form one or more internal voids. Vesicles may be formulated, for example, from a stabilizing material such as a lipid, including the various lipids described herein, a proteinaceous material, including the various proteins described herein, a polymeric material, including the various polymeric materials described herein, and a surfactant, including the various surfactants described herein. As discussed herein, vesicles may also be formulated from carbohydrates and other stabilizing materials, as desired. The lipids, proteins, polymers, surfactants, and/or other vesicle forming stabilizing materials may be natural, synthetic or semi-synthetic. Preferred vesicles are those which comprise walls or membranes formulated from lipids or surfactants. The walls or membranes may be concentric or otherwise. The stabilizing compounds may be in the form of one or more monolayers or bilayers. In the case of more than one monolayer or bilayer, the monolayers or bilayers may be concentric. Stabilizing compounds may be used to form a unilamellar vesicle (comprised of one monolayer or bilayer), an oligolamellar vesicle (comprised of about two or about three monolayers or bilayers) or a multilamellar vesicle (comprised of more than about three monolayers or bilayers). The walls or membranes of vesicles may be substantially solid (uniform), or they may be porous or semi-porous. The vesicles described herein include such entities commonly referred to as, for example, liposomes, micelles, bubbles, microbubbles, microspheres, lipid-coated bubbles, polymer-coated bubbles and/or protein-coated bubbles, microbubbles and/or microspheres, nanospheres, microballoons, microcapsules, aerogels, clathrate bound vesicles, hexagonal H II phase structures, and the like. The internal void of the vesicles may be filled with water, oil, liquids, gases, gaseous precursors and bioactive agents, if desired, and/or other materials. The vesicles may also comprise a targeting ligand, if desired.

Brief Summary Text (37):
"Diagnostic agent" refers to any agent which may be used in connection with methods for imaging an internal region of a patient and/or diagnosing the presence or absence of a disease in a patient. Exemplary diagnostic agents include, for example, contrast agents for use in connection with ultrasound imaging, magnetic resonance imaging or computed tomography imaging of a patient. Diagnostic agents may also include any other agents useful in facilitating diagnosis of a disease or other condition in a patient, whether or not imaging methodology is employed. As defined herein, a "diagnostic agent" is also a type of bioactive agent.

Brief Summary Text (100):
In the compound of formula (I), D may be a wide variety of bioactive agents. Suitable bioactive agents for use in the prodrugs of the present invention include, for example, antineoplastic agents, blood products, biological response modifiers, anti-fungal agents, hormones, steroids, vitamins, peptides, peptide analogs, enzymes, anti-allergenic agents, anti-coagulation agents, circulatory agents, anti-tubercular agents, anti-viral agents, anti-anginal agents, antibiotics, anti-inflammatory agents, analgesics, anti-protozoan agents, anti-rheumatic agents, narcotics, cardiac glycoside agents, chelates, neuromuscular blocking agents, sedatives (hypnotics), local anesthetic agents, general anesthetic agents, radioactive particles, radioactive ions, X-ray contrast agents, monoclonal

antibodies, polyclonal antibodies and genetic material. In view of the present disclosure, one skilled in the art could determine whether any particular bioactive agent could be used in the compounds of the present invention. Examples of suitable bioactive agents are listed below; however, the list is exemplary only and is not intended to limit the bioactive agents that may be used in the present invention.

Brief Summary Text (127):
X-ray contrast agents, include, for example, X-ray contrast agents known in the art that contain heavy metals such as yttrium, ytterbium, lanthanides in chelates or other iodinated materials, such as iothalamate.

Brief Summary Text (161):
Exemplary lipids which may be used to prepare the stabilizing materials of the present invention include, for example, fatty acids, lysolipids, fluorinated lipids, phosphocholines, such as those associated with platelet activation factors (PAF) (Avanti Polar Lipids, Alabaster, Ala.), including 1-alkyl-2-acetoyl-sn-glycero 3-phosphocholines, and 1-alkyl-2-hydroxy-sn-glycero 3-phosphocholines, which target blood clots; phosphatidylcholine with both saturated and unsaturated lipids, including dioleoyphosphatidylcholine; dimyristoylphosphatidylcholine; dipentadecanoylphosphatidylcholine; dilauroylphosphatidylcholine; dipalmitoylphosphatidylcholine (DPPC); distearoylphosphatidylcholine (DSPC); and diarachidonylphosphatidylcholine (DAPC); phosphatidylethanolamines, such as dioleoylphosphatidylethanolamine, dipalmitoylphosphatidylethanolamine (DPPE) and distearoylphosphatidylethanolamine (DSPE); phosphatidylserine; phosphatidylglycerols, including distearoylphosphatidylglycerol (DSPG); phosphatidylinositol; sphingolipids such as sphingomyelin; glycolipids such as ganglioside GM1 and GM2; glucolipids; sulfatides; glycosphingolipids; phosphatidic acids, such as dipalmitoylphosphatidic acid (DPPA) and distearoylphosphatidic acid (DSPA); palmitic acid; stearic acid; arachidonic acid; oleic acid; lipids bearing polymers, such as chitin, hyaluronic acid, polyvinylpyrrolidone or polyethylene glycol (PEG), also referred to herein as "pegylated lipids" with preferred lipid bearing polymers including DPPE-PEG (DPPE-PEG), which refers to the lipid DPPE having a PEG polymer attached thereto, including, for example, DPPE-PEG5000, which refers to DPPE having attached thereto a PEG polymer having a mean average molecular weight of about 5000; lipids bearing sulfonated mono-, di-, oligo- or polysaccharides; cholesterol, cholesterol sulfate and cholesterol hemisuccinate; tocopherol hemisuccinate; lipids with ether and ester-linked fatty acids; polymerized lipids (a wide variety of which are well known in the art); diacetyl phosphate; dicetyl phosphate; stearylamine; cardiolipin; phospholipids with short chain fatty acids of about 6 to about 8 carbons in length; synthetic phospholipids with asymmetric acyl chains, such as, for example, one acyl chain of about 6 carbons and another acyl chain of about 12 carbons; ceramides; non-ionic liposomes including niosomes such as polyoxyalkylene (e.g., polyoxyethylene) fatty acid esters, polyoxyalkylene (e.g., polyoxyethylene) fatty alcohols, polyoxyalkylene (e.g., polyoxyethylene) fatty alcohol ethers, polyoxyalkylene (e.g., polyoxyethylene) sorbitan fatty acid esters (such as, for example, the class of compounds referred to as TWEEN.RTM., including, for example, TWEEN.RTM. 20, TWEEN.RTM. 40 and TWEEN.RTM. 80, commercially available from ICI Americas, Inc., Wilmington, Del.), glycerol polyethylene glycol oxystearate, glycerol polyethylene glycol ricinoleate, alkyloxylated (e.g., ethoxylated) soybean sterols, alkyloxylated (e.g., ethoxylated) castor oil, polyoxyethylene-polyoxypropylene polymers, and polyoxyalkylene (e.g., polyoxyethylene) fatty acid stearates; sterol aliphatic acid esters including cholesterol sulfate, cholesterol butyrate, cholesterol isobutyrate, cholesterol palmitate, cholesterol stearate, lanosterol acetate, ergosterol palmitate, and phytosterol n-butyrate; sterol esters of sugar acids including cholesterol glucuronide, lanosterol glucuronide, 7-dehydrocholesterol glucuronide, ergosterol glucuronide, cholesterol gluconate, lanosterol gluconate, and ergosterol gluconate; esters of sugar acids and alcohols including lauryl glucuronide, stearoyl glucuronide, myristoyl glucuronide, lauryl gluconate, myristoyl gluconate, and stearoyl gluconate; esters of sugars and aliphatic acids including sucrose laurate, fructose laurate, sucrose palmitate, sucrose stearate, glucuronic acid, gluconic acid and polyuronic acid; saponins including sarsasapogenin, smilagenin, hederagenin, oleanolic acid, and digitoxigenin; glycerol dilaurate, glycerol trilaurate, glycerol dipalmitate, glycerol and glycerol esters including glycerol tripalmitate, glycerol distearate, glycerol tristearate, glycerol dimyristate,

glycerol trimyristate; long chain alcohols including n-decyl alcohol, lauryl alcohol, myristyl alcohol, cetyl alcohol, and n-octadecyl alcohol; 6-(5-cholesten-3.beta.-yloxy)-1-thio-.beta.-D-galactopyranoside; digalactosyldiglyceride; 6-(5-cholesten-3.beta.-yloxy)-hexyl-6-amino-6-deoxy-1-thio-.beta.-D-galact opyranoside; 6-(5-cholesten-3.beta.-yloxy)hexyl-6-amino-6-deoxyl-1-thio-.alpha.-D-manno pyranoside; 12-(((7'-diethylaminocoumarin-3-yl)-carbonyl)methylamino)octadecanoic acid; N-[12-(((7'-diethylaminocoumarin-3-yl)-carbonyl)methylamino)octadeca noyl]-2-aminopalmitic acid; cholesteryl(4'-trimethyl-ammonio)butanoate; N-succinyldioleoylphosphatidylethanolamine; 1,2-dioleoylsn-glycerol; 1,2-dipalmitoyl-sn-3-succinylglycerol; 1,3-dipalmitoyl-2-succinylglycerol; 1-hexadecyl-2-palmitoylglycerophosphoethanolamine and palmitoylhomocysteine, and/or any combinations thereof In preferred embodiments, the stabilizing materials comprise phospholipids, including one or more of DPPC, DPPE, DPPA, DSPC, DSPE, DSPG, and DAPC.

Brief Summary Text (177):
In addition to stabilizing materials and/or vesicles formulated from lipids and/or proteins, embodiments of the present invention may also involve stabilizing materials or vesicles formulated from polymers which may be of natural, semi-synthetic (modified natural) or synthetic origin. Polymer denotes a compound comprised of two or more repeating monomeric units, and preferably 10 or more repeating monomeric units. Semi-synthetic polymer (or modified natural polymer) denotes a natural polymer that has been chemically modified in some fashion. Examples of suitable natural polymers include naturally occurring polysaccharides, such as, for example, arabinans, fructans, fucans, galactans, galacturonans, glucans, mannans, xylans (such as, for example, inulin), levan, fucoidan, carrageenan, galatocarolose, pectic acid, pectins, including amylose, pullulan, glycogen, amylopectin, cellulose, dextran, dextrin, dextrose, glucose, polyglucose, polydextrose, pustulan, chitin, agarose, keratin, chondroitin, dermatan, hyaluronic acid, alginic acid, xanthin gum, starch and various other natural homopolymer or heteropolymers, such as those containing one or more of the following aldoses, ketoses, acids or amines: erythrose, threose, ribose, arabinose, xylose, lyxose, allose, altrose, glucose, dextrose, mannose, gulose, idose, galactose, talose, erythrulose, ribulose, xylulose, psicose, fructose, sorbose, tagatose, mannitol, sorbitol, lactose, sucrose, trehalose, maltose, cellobiose, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, glucuronic acid, gluconic acid, glucaric acid, galacturonic acid, mannuronic acid, glucosamine, galactosamine, and neuraminic acid, and naturally occurring derivatives thereof. Accordingly, suitable polymers include, for example, proteins, such as albumin or polylactide-coglycolide polymers, and polyalginates. Exemplary semi-synthetic polymers include carboxymethylcellulose, hydroxymethylcellulose, hydroxypropylmethylcellulose, methylcellulose, and methoxycellulose. Exemplary synthetic polymers suitable for use in the present invention include polyphosphazenes, hydroxyapatite polymers, fluoroapatite polymers, polyethylenes (such as, for example, polyethylene glycol (including, for example, the class of compounds referred to as Pluronics.RTM., commercially available from BASF, Parsippany, N.J.), polyoxyethylene, and polyethylene terephthlate), polypropylenes (such as, for example, polypropylene glycol), polyurethanes (such as, for example, polyvinyl alcohol (PVA), polyvinyl chloride and polyvinylpyrrolidone), polyamides including nylon, polystyrene, polylactic acids, fluorinated hydrocarbon polymers, fluorinated carbon polymers (such as, for example, polytetrafluoroethylene), acrylate, methacrylate, and polymethylmethacrylate, and derivatives thereof. Preferred are synthetic polymers or copolymers prepared from monomers, such as acrylic acid, methacrylic acid, ethyleneimine, crotonic acid, acrylamide, ethyl acrylate, methyl methacrylate, 2-hydroxyethyl methacrylate (HEMA), lactic acid, glycolic acid, .epsilon.-caprolactone, acrolein, cyanoacrylate, bisphenol A, epichlorhydrin, hydroxyalkyl-acrylates, siloxane, dimethylsiloxane, ethylene oxide, ethylene glycol, hydroxyalkyl-methacrylates, N-substituted acrylamides, N-substituted methacrylamides, N-vinyl-2-pyrrolidone, 2,4-pentadiene-1-ol, vinyl acetate, acrylonitrile, styrene, p-amino-styrene, p-amino-benzyl-styrene, sodium styrene sulfonate, sodium 2-sulfoxyethyl-methacrylate, vinyl pyridine, aminoethyl methacrylates, 2-methacryloyloxy-trimethylammonium chloride, and polyvinylidene, as well

polyfunctional crosslinking monomers such as N,N'-methylenebisacrylamide, ethylene glycol dimethacrylates, 2,2'-(p-phenylenedioxy)-diethyl dimethacrylate, divinylbenzene, triallylamine and methylenebis-(4-phenylisocyanate), including combinations thereof. Preferable polymers include polyacrylic acid, polyethyleneimine, polymethacrylic acid, polymethylmethacrylate, polysiloxane, polydimethylsiloxane, polylactic acid, poly(.epsilon.-caprolactone), epoxy resin, poly(ethylene oxide), poly(ethylene glycol), and polyamide (nylon) polymers. Preferable copolymers include the following: polyvinylidene-polyacrylonitrile, polyvinylidene-polyacrylonitrile-polymethylmethacrylate, polystyrene-polyacrylonitrile and poly d-1, lactide co-glycolide polymers. A preferred copolymer is polyvinylidene-polyacrylonitrile. Other suitable biocompatible monomers and polymers will be apparent to one skilled in the art in view of the present disclosure.

Brief Summary Text (179):
It is not always possible to determine whether a given material is a basic or an auxiliary agent, since the functioning of the material is determined empirically, for example, by the results produced with respect to producing stabilized materials or vesicles. As an example of how the basic and auxiliary materials may function, it has been observed that the simple combination of a biocompatible lipid and water or saline when shaken will often give a cloudy solution subsequent to autoclaving for sterilization. Such a cloudy solution may function as a contrast agent, but is aesthetically objectionable and may imply instability in the form of undissolved or undispersed lipid particles. Cloudy solutions may also be undesirable where the undissolved particulate matter has a diameter of greater than about 7 .mu.m, and especially greater than about 10 .mu.m. Manufacturing steps, such as sterile filtration, may also be problematic with solutions which contain undissolved particulate matter. Thus, propylene glycol may be added to remove this cloudiness by facilitating dispersion or dissolution of the lipid particles. Propylene glycol may also function as a wetting agent which can improve vesicle formation and stabilization by increasing the surface tension on the vesicle membrane or skin. It is possible that propylene glycol can also function as an additional layer that may coat the membrane or skin of the vesicle, thus providing additional stabilization. The conventional surfactants described by D'Arrigo, U.S. Pat. Nos. 4,684,479 and 5,215,680, the disclosures of each of which are hereby incorporated by reference herein in their entirety, may be used as basic or auxiliary stabilizing materials in the present invention.

Brief Summary Text (195):
Polymers useful as stabilizing materials and for preparing the gas and/or gaseous precursor filled vesicles may be of natural, semi-synthetic (modified natural) or synthetic origin. Exemplary natural polymers include naturally occurring polysaccharides, such as, for example, arabinans, fructans, fucans, galactans, galacturonans, glucans, mannans, xylans (such as, for example, inulin), levan, fucoidan, carrageenan, galatocarolose, pectic acid, pectins, including amylose, pullulan, glycogen, amylopectin, cellulose, dextran, dextrin, dextrose, glucose, polyglucose, polydextrose, pustulan, chitin, agarose, keratin, chondroitin, dermatan, hyaluronic acid, alginic acid, xanthin gum, starch and various other natural homopolymer or heteropolymers, such as those containing one or more of the following aldoses, ketoses, acids or amines: erythose, threose, ribose, arabinose, xylose, lyxose, allose, altrose, glucose, dextrose, mannose, gulose, idose, galactose, talose, erythrulose, ribulose, xylulose, psicose, fructose, sorbose, tagatose, mannitol, sorbitol, lactose, sucrose, trehalose, maltose, cellobiose, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, glucuronic acid, gluconic acid, glucaric acid, galacturonic acid, mannuronic acid, glucosamine, galactosamine, and neuraminic acid, and naturally occurring derivatives thereof Accordingly, suitable polymers include, for example, proteins, such as albumin, polyalginates, and polylactide-coglycolide polymers. Exemplary semi-synthetic polymers include carboxymethylcellulose, hydroxymethylcellulose, hydroxypropylmethylcellulose, methylcellulose, and methoxycellulose. Exemplary synthetic polymers include polyphosphazenes, hydroxyapatites, fluoroapatite polymers, polyethylenes (such as, for example, polyethylene glycol (including for example, the class of compounds referred to as Pluronics.RTM., commercially available from BASF, Parsippany, N.J.), polyoxyethylene, and polyethylene terephthlate), polypropylenes (such as, for

example, polypropylene glycol), polyurethanes (such as, for example, polyvinyl alcohol (PVA), polyvinyl chloride and polyvinylpyrrolidone), polyamides including nylon, polystyrene, polylactic acids, fluorinated hydrocarbon polymers, fluorinated carbon polymers (such as, for example, polytetrafluoroethylene), acrylate, methacrylate, and polymethylmethacrylate, and derivatives thereof. Methods for the preparation of vesicles which employ polymers as stabilizing compounds will be readily apparent to one skilled in the art, in view of the present disclosure, when coupled with information known in the art, such as that described and referred to in Unger, U.S. Pat. No. 5,205,290, the disclosure of which is hereby incorporated by reference herein in its entirety.

Brief Summary Text (196):
Preferred embodiments of the present invention involve vesicles which comprise three components: (1) a neutral lipid, for example, a nonionic or zwitterionic lipid, (2) a negatively charged lipid, and (3) a lipid bearing a stabilizing material, for example, a hydrophilic polymer. Preferably, the amount of the negatively charged lipid will be greater than about 1 mole percent of the total lipid present, and the amount of lipid bearing a hydrophilic polymer will be greater than about 1 mole percent of the total lipid present. Exemplary and preferred negatively charged lipids include phosphatidic acids. The lipid bearing a hydrophilic polymer will desirably be a lipid covalently linked to the polymer, and the polymer will preferably have a weight average molecular weight of from about 400 to about 100,000. Suitable hydrophilic polymers are preferably selected from the group consisting of polyethylene glycol (PEG), polypropylene glycol, polyvinylalcohol, and polyvinylpyrrolidone and copolymers thereof, with PEG polymers being preferred.

Brief Summary Text (198):
In some embodiments of the present invention, the lipid compositions may include about 77.5 mol % DPPC, 12.5 mol % of DPPA, and 10 mol % of DPPE-PEG5000. Also preferred are compositions which comprise about 80 to about 90 mole % DPPC, about 5 to about 15 mole % DPPA and about 5 to about 15 mole % DPPE-PEG5000. Especially preferred are compositions which comprise DPPC, DPPA and DPPE-PEG5000 in a mole % ratio of 82:10:8, respectively. DPPC is substantially neutral, since the phosphatidyl portion is negatively charged and the choline portion is positively charged. Consequently, DPPA, which is negatively charged, may be added to enhance stabilization in accordance with the mechanism described above. DPPE-PEG provides a pegylated material bound to the lipid membrane or skin of the vesicle by the DPPE moiety, with the PEG moiety free to surround the vesicle membrane or skin, and thereby form a physical barrier to various enzymatic and other endogenous agents in the body whose function is to degrade such foreign materials. The DPPE-PEG may provide more vesicles of a smaller size which are safe and stable to pressure when combined with other lipids, such as DPPC and DPPA, in the given ratios. It is also theorized that the pegylated material, because of its structural similarity to water, may be able to defeat the action of the macrophages of the human immune system, which would otherwise tend to surround and remove the foreign object. The result is an increase in the time during which the stabilized vesicles may function as diagnostic imaging contrast media.

Brief Summary Text (200):
The gas and/or gaseous precursor filled vesicles used in the present invention may be controlled according to size, solubility and heat stability by choosing from among the various additional or auxiliary stabilizing materials described herein. These materials can affect the parameters of the vesicles, especially vesicles formulated from lipids, not only by their physical interaction with the membranes, but also by their ability to modify the viscosity and surface tension of the surface of the gas and/or gaseous precursor filled vesicle. Accordingly, the gas and/or gaseous precursor filled vesicles used in the present invention may be favorably modified and further stabilized, for example, by the addition of one or more of a wide variety of (i) viscosity modifiers, including, for example, carbohydrates and their phosphorylated and sulfonated derivatives; polyethers, preferably with molecular weight ranges between 400 and 100,000; and di- and trihydroxy alkanes and their polymers, preferably with molecular weight ranges between 200 and 50,000; (ii) emulsifying and/or solubilizing agents including, for example, acacia, cholesterol, diethanolamine, glyceryl monostearate, lanolin alcohols, lecithin, mono- and di-glycerides, mono-ethanolarnine, oleic acid, oleyl alcohol, poloxamer, for

example, poloxamer 188, poloxamer 184, and poloxamer 181, Pluronics.RTM. (BASF, Parsippany, N.J.), polyoxyethylene 50 stearate, polyoxyl 35 castor oil, polyoxyl 10 oleyl ether, polyoxyl 20 cetostearyl ether, polyoxyl 40 stearate, polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, propylene glycol diacetate, propylene glycol monostearate, sodium lauryl sulfate, sodium stearate, sorbitan monolaurate, sorbitan monooleate, sorbitan monopalmitate, sorbitan monostearate, stearic acid, trolamine, and emulsifying wax; (iii) suspending and/or viscosity-increasing agents, including, for example, acacia, agar, alginic acid, aluminum monostearate, bentonite, magma, carbomer 934P, carboxymethylcellulose, calcium and sodium and sodium 12, carrageenan, cellulose, dextran, gelatin, guar gum, locust bean gum, veegum, hydroxyethyl cellulose, hydroxypropyl methylcellulose, magnesium-aluminum-silicate, Zeolites.RTM., methylcellulose, pectin, polyethylene oxide, povidone, propylene glycol alginate, silicon dioxide, sodium alginate, tragacanth, xanthin gum, .alpha.-d-gluconolactone, glycerol and mannitol; (iv) synthetic suspending agents, such as polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), polyvinylalcohol (PVA), polypropylene glycol (PPG), and polysorbate; and (v) tonicity raising agents which stabilize and add tonicity, including, for example, sorbitol, mannitol, trehalose, sucrose, propylene glycol and glycerol.

Brief Summary Text (201):
The present lipid and/or vesicles are desirably formulated in an aqueous environment which can induce the lipid, because of its hydrophobic-hydrophilic nature, to form vesicles, which may be the most stable configuration which can be achieved in such an environment. Diluents which may be used to create such an aqueous environment include, for example, water, normal saline, physiological saline, deionized water and water containing one or more dissolved solutes, such as salts or sugars, which preferably do not interfere with the formation and/or stability of the vesicles or their use as delivery vehicles or diagnostic agents, such as ultrasound contrast agents, MRI contrast agents, CT contrast agents and optical imaging agents.

Brief Summary Text (202):
The present stabilizing materials or vesicles preferably comprise a gas, such as an inert gas. The gas provides the stabilizing materials or vesicles with enhanced reflectivity, particularly in connection with stabilizing materials or vesicles in which the gas is entrapped within the stabilizing materials or vesicles. This may increase their effectiveness as delivery vehicles or contrast agents. Preferred gases are inert and biocompatible, and include, for example, air, noble gases, such as helium, rubidium hyperpolarized xenon, hyperpolarized argon, hyperpolarized helium, neon, argon and xenon, carbon dioxide, nitrogen, fluorine, oxygen, sulfur-based gases, such as sulfur hexafluoride and sulfur tetrafluoride, fluorinated gases, including, for example, partially fluorinated gases or completely fluorinated gases, and mixtures thereof. Paramagnetic gases, such as .sup.17 O.sub.2 may also be used in the stabilizing materials and vesicles.

Brief Summary Text (249):
In accordance with preferred embodiments, the targeting ligands may be linked or attached to the prodrugs, lipids, proteins, polymers, or surfactants or other stabilizing materials via a linking group. A variety of linking groups are available and would be apparent to one skilled in the art in view of the present disclosure. Preferably, the linking group comprises a hydrophilic polymer. Suitable hydrophilic linker polymers include, for example, polyalkyleneoxides such as, for example, polyethylene glycol (PEG) and polypropylene glycol (PPG), polyvinylpyrrolidones, polyvinylmethylethers, polyacrylamides, such as, for example, polymethacrylamides, polydimethylacrylamides and polyhydroxypropylmethacrylamides, polyhydroxyethyl acrylates, polyhydroxypropyl methacrylates, polymethyloxazolines, polyethyloxazolines, polyhydroxyethyloxazolines, polyhyhydroxypropyloxazolines, polyvinyl alcohols, polyphosphazenes, poly(hydroxyalkylcarboxylic acids), polyoxazolidines, polyaspartamide, and polymers of sialic acid (polysialics). The hydrophilic polymers are preferably selected from the group consisting of PEG, PPG, polyvinylalcohol and polyvinylpyrrolidone and copolymers thereof, with PEG and PPG polymers being more preferred and PEG polymers being even more prefered. Thus, in embodiments involving lipid compositions which comprise lipids bearing polymers including, for example, DPPE-PEG, the targeting ligand may be linked directly to the polymer which is attached to the lipid to provide, for example, a conjugate of DPPE-PEG-TL, where TL is a targeting ligand. Thus, using the example DPPE-PEG, such

as, for example, DPPE-PEG5000, the aforementioned conjugate may be represented as DPPE-PEG5000-TL. The hydrophilic polymer used as a linking group is preferably a bifunctional polymer, for example, bifunctional PEG, such as diamino-PEG. In this case, one end of the PEG group is linked, for example, to a lipid compound, and is bound at the free end to the targeting ligand via an amide linkage. A hydrophilic polymer, for example, PEG, substituted with a terminal carboxylate group on one end and a terminal amino group on the other end, may also be used. These latter bifunctional hydrophilic polymer may be preferred since they possess various similarities to amino acids.

Brief Summary Text (260):
In preferred embodiments of the present invention, the targeted compounds, namely, targeted stabilizing materials, including prodrugs, lipids, proteins, polymers and surfactants, are incorporated in compositions which are used to form targeted emulsions and/or targeted vesicles, including, for example, targeted emulsions, targeted micelles, targeted liposomes, targeted albumin coated microspheres, and/or targeted polymer coated microspheres. The targeting ligand which is attached to the compounds from which the vesicles are prepared may be directed, for example, outwardly from the surface of the vesicle. Thus, there is provided a targeted vesicle which can be used to target receptors and tissues.

Brief Summary Text (269):
In the above compounds, P is a hydrophilic polymer. Preferably, P is a hydrophilic polymer selected from the group consisting of polyalkyleneoxides, polyvinyl alcohol, polyvinylpyrrolidones, polyacrylamides, polymethacrylamides, polyphosphazenes, phosphazene, poly(hydroxyalkylcarboxylic acids) and polyoxazolidines. More preferably, P is a polyalkyleneoxide polymer, with polyethylene glycol and polypropylene glycol being even more preferred and polyethylene glycol being particularly preferred.

Brief Summary Text (279):
In the above formula, Z is a hydrophilic polymer. Preferably, Z is selected from the group consisting of polyalkyleneoxides, polyvinyl alcohol, polyvinylpyrrolidones, polyacrylamides, polymethacrylamides, polyphosphazenes, poly(hydroxyalkylcarboxylic acids) and polyoxazolidines. More preferably, Z comprises a polyalkyleneoxide. Even more preferably, Z is a polyalkyleneoxide selected from the group consisting of polyethylene glycol and polypropylene glycol, with polyethylene glycol being still more preferred. In certain other preferred embodiments, Z is a hydrophilic polymer other than polyalkyleneoxides, including polyethylene glycol and polypropylene glycol. The molecular weight of Z may vary, depending, for example, on the particular end-use of the compounds. Preferably, Z is a polymer having a molecular weight which ranges from about 100 to about 10,000, and all combinations and subcombinations of ranges therein. More preferably, Z is a polymer having a molecular weight of from about 1,000 to about 5,000. Also preferred are polymers which exhibit polydispersities ranging from greater than about 1 to about 3, and all combinations and subcombinations of ranges therein. More preferably, Z is a polymer having a polydispersity of from greater than about 1 to about 2, with polydispersities of from greater than about 1 to about 1.5 being even more preferred, and polydispersities of from greater than about 1 to about 1.2 being still more preferred.

Brief Summary Text (314):
Alternatively, the gaseous precursors may be utilized to create stable gas filled vesicles which are pre-formed prior to use. In this embodiment, the gaseous precursor is added to a container housing a lipid composition at a temperature below the liquid-gaseous phase transition temperature of the respective gaseous precursor. As the temperature is increased, and an emulsion is formed between the gaseous precursor and liquid solution, the gaseous precursor undergoes transition from the liquid to the gaseous state. As a result of this heating and gas formation, the gas displaces the air in the head space above the liquid mixture so as to form gas filled vesicles which entrap the gas of the gaseous precursor, ambient gas (e.g. air), or coentrap gas state gaseous precursor and ambient air. This phase transition can be used for optimal mixing and formation of the contrast agent. For example, the gaseous precursor, perfluorobutane, can be entrapped in the lipid vesicles and as the temperature is raised beyond the boiling point of perfluorobutane (4.degree.

C.), perfluorobutane gas is entrapped in the vesicles.

Brief Summary Text (336):
Conventional, aqueous-filled liposomes of the prior art are routinely formed at a temperature above the phase transition temperature of the lipids used to make them, since they are more flexible and thus useful in biological systems in the liquid crystalline state. See, for example, Szoka and Papahadjopoulos, Proc. Natl. Acad. Sci. (1978) 75, 4194-4198. In contrast, the vesicles made according to certain preferred embodiments described herein are gaseous precursor filled, which imparts greater flexibility, since gaseous precursors after gas formation are more compressible and compliant than an aqueous solution.

Brief Summary Text (342):
Polyethylene glycol containing fragments, that may be used in the above schemes include, for example, PEG2-NHS ester, NHS-PEG-VS, NHS-PEG-MAL, methoxy-PEG-vinylsulfone, PEG-(VS).sub.2, methoxy-PEG-ald, PEG-(ald).sub.2, methoxy-PEG-epx, PEG-(epx).sub.2, methoxy-PEG-Tres, PEG-(Tres).sub.2, methoxy-PEG-NPC, PEG-(NPC).sub.2, methoxy-PEG-CDI, PEG-(CDI).sub.2, mPEG-Gly-OSu, mPEG-NLe-OSu, methoxy-SPA-PEG, (SPA).sub.2 -PEG, methoxy-SS-PEG, (SS).sub.2 -PEG all of which are available from Shearwater PoAlabas, Inc. (Huntsville, Ala.). Where these types of fragments are used, i.e., where the fragments may not themselves have surfactant properties adequate for a given ultrasound contrast formulation, or act only weakly as surfactants, the conjugate formed can be used in conjunction with other surfactants in the final formulation.

Brief Summary Text (346):
It may be preferable to carry out the sonication in such a manner to produce foaming, and especially intense foaming, of the solution. Generally speaking, intense foaming and aerosolating are important for obtaining a contrast agent having enhanced concentration and stability. To promote foaming, the power input to the sonicator horn may be increased, and the process may be operated under mild pressure, for example, about 1 to about 5 psi. Foaming may be easily detected by the cloudy appearance of the solution, and by the foam produced.

Brief Summary Text (347):
Suitable methods for the preparation of protein-based vesicles may also involve physically or chemically altering the protein or protein derivative in aqueous solution to denature or fix the material. For example, protein-based vesicles may be prepared from a 5% aqueous solution of HSA by heating after formation or during formation of the contrast agent via sonication. Chemical alteration may involve chemically denaturing or fixing by binding the protein with a difunctional aldehyde, such as gluteraldehyde. For example, the vesicles may be reacted with 0.25 grams of 50% aqueous gluteradehyde per gram of protein at pH 4.5 for 6 hours. The unreacted gluteraldehyde may then be washed away from the protein.

Brief Summary Text (383):
In the case of diagnostic applications, such as ultrasound and CT, energy, such as ultrasonic energy, is applied to at least a portion of the patient to image the target tissue. A visible image of an internal region of the patient is then obtained, such that the presence or absence of diseased tissue can be ascertained. With respect to ultrasound, ultrasonic imaging techniques, including second harmonic imaging, and gated imaging, are well known in the art, and are described, for example, in Uhlendorf, "Physics of Ultrasound Contrast Imaging: Scattering in the Linear Range", IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control, Vol. 14(1), pp. 70-79 (1994) and Sutherland, et al., "Color Doppler Myocardial Imaging: A New Technique for the Assessment of Myocardial Function", Journal of the American Society of Echocardiography, Vol. 7(5), pp. 441-458 (1994), the disclosures of each of which are hereby incorporated herein by reference in their entirety.

Brief Summary Text (384):
Ultrasound can be used for both diagnostic and therapeutic purposes. In diagnostic ultrasound, ultrasound waves or a train of pulses of ultrasound may be applied with a transducer. The ultrasound is generally pulsed rather than continuous, although it may be continuous, if desired. Thus, diagnostic ultrasound generally involves the

application of a pulse of echoes, after which, during a listening period, the ultrasound transducer receives reflected signals. Harmonics, ultraharmonics or subharmonics may be used. The second harmonic mode may be beneficially employed, in which the 2x frequency is received, where x is the incidental frequency. This may serve to decrease the signal from the background material and enhance the signal from the transducer using the targeted contrast media of the present invention which may be targeted to the desired site, for example, blood clots. Other harmonic signals, such as odd harmonics signals, for example, 3x or 5x, would be similarly received using this method. Subharmonic signals, for example, x/2 and x/3, may also be received and processed so as to form an image.

Brief Summary Text (404):
Other preferred therapeutics include genetic material such as nucleic acids, RNA, and DNA, of either natural or synthetic origin, including recombinant RNA and DNA and antisense RNA and DNA. Types of genetic material that may be used include, for example, genes carried on expression vectors such as plasmids, phagemids, cosmids, yeast artificial chromosomes (YACs), and defective or "helper" viruses, antigene nucleic acids, both single and double stranded RNA and DNA and analogs thereof, such as phosphorothioate and phosphorodithioate oligodeoxynucleotides. Additionally, the genetic material may be combined, for example, with proteins or other polymers. Examples of genetic therapeutics that may be applied using the liposomes of the present invention include DNA encoding at least a portion of an HLA gene, DNA encoding at least a portion of dystrophin, DNA encoding at least a portion of CFTR, DNA encoding at least a portion of IL-2, DNA encoding at least a portion of TNF, an antisense oligonucleotide capable of binding the DNA encoding at least a portion of Ras.

Brief Summary Text (409):
As discussed above, the delivery vehicles and stabilizing materials of the present invention may be used in connection with diagnostic imaging, therapeutic imaging and drug delivery, including, for example, ultrasound (US), magnetic resonance imaging (I), nuclear magnetic resonance (NMR), computed tomography (CT), electron spin resonance (ESR), nuclear medical imaging, optical imaging, elastography, drug delivery with ultrasound, radiofrequency (RF) and microwave laser. The delivery vehicles and stabilizing materials of the present invention may be used in combination with various contrast agents, including conventional contrast agents, which may serve to increase their effectiveness as contrast agents for diagnostic and therapeutic imaging.

Brief Summary Text (410):
Examples of suitable contrast agents for use in combination with the present stabilizing materials include, for example, stable free radicals, such as, stable nitroxides, as well as compounds comprising transition, lanthanide and actinide elements, which may, if desired, be in the form of a salt or may be covalently or non-covalently bound to complexing agents, including lipophilic derivatives thereof, or to proteinaceous macromolecules. Preferable transition, lanthanide and actinide elements include, for example, Gd(III), Mn(II), Cu(II), Cr(III), Fe(II), Fe(III), Co(II), Er(II), Ni(II), Eu(III) and Dy(III). More preferably, the elements may be Gd(III), Mn(II), Cu(II), Fe(II), Fe(III), Eu(III) and Dy(III), most preferably Mn(II) and Gd(III). The foregoing elements may be in the form of a salt, including inorganic salts, such as a manganese salt, for example, manganese chloride, manganese carbonate, manganese acetate, and organic salts, such as manganese gluconate and manganese hydroxylapatite. Other exemplary salts include salts of iron, such as iron sulfides, and ferric salts, such as ferric chloride.

Brief Summary Text (412):
Nitroxides are paramagnetic contrast agents which increase both T1 and T2 relaxation rates on MRI by virtue of the presence of an unpaired electron in the nitroxide molecule. As known to one of ordinary skill in the art, the paramagnetic effectiveness of a given compound as an MRI contrast agent may be related, at least in part, to the number of unpaired electrons in the paramagnetic nucleus or molecule, and specifically, to the square of the number of unpaired electrons. For example, gadolinium has seven unpaired electrons whereas a nitroxide molecule has one unpaired electron. Thus, gadolinium is generally a much stronger MRI contrast agent than a nitroxide. However, effective correlation time, another important

parameter for assessing the effectiveness of contrast agents, confers potential increased relaxivity to the nitroxides. When the tumbling rate is slowed, for example, by attaching the paramagnetic contrast to a large molecule, it will tumble more slowly and thereby more effectively transfer energy to hasten relaxation of the water protons. In gadolinium, however, the electron spin relaxation time is rapid and will limit the extent to which slow rotational correlation times can increase relaxivity. For nitroxides, however, the electron spin correlation times are more favorable and tremendous increases in relaxivity may be attained by slowing the rotational correlation time of these molecules. The gas filled vesicles of the present invention are ideal for attaining the goals of slowed rotational correlation times and resultant improvement in relaxivity. Although not intending to be bound by any particular theory of operation, it is contemplated that since the nitroxides may be designed to coat the perimeters of the vesicles, for example, by making alkyl derivatives thereof, the resulting correlation times can be optimized. Moreover, the resulting contrast medium of the present invention may be viewed as a magnetic sphere, a geometric configuration which maximizes relaxivity.

Brief Summary Text (413):
Exemplary superparamagnetic contrast agents suitable for use in the compositions of the present invention include metal oxides and sulfides which experience a magnetic domain, ferro- or ferrimagnetic compounds, such as pure iron, magnetic iron oxide, such as magnetite, .gamma.-Fe.sub.2 O.sub.3, Fe.sub.3 O.sub.4, manganese ferrite, cobalt ferrite and nickel ferrite. Paramagnetic gases can also be employed in the present compositions, such as oxygen 17 gas (.sup.17 O.sub.2). In addition, hyperpolarized xenon, neon, or helium gas may also be employed. MR whole body imaging may then be employed to rapidly screen the body, for example, for thrombosis, and ultrasound may be applied, if desired, to aid in thrombolysis.

Brief Summary Text (414):
The contrast agents, such as the paramagnetic and superparamagnetic contrast agents described above, may be employed as a component within the delivery vehicles and/or stabilizing materials. With respect to vesicles, the contrast agents may be entrapped within the internal void thereof, administered as a solution with the vesicles, incorporated with any additional stabilizing materials, or coated onto the surface or membrane of the vesicle. Mixtures of any one or more of the paramagnetic agents and/or superparamagnetic agents in the present compositions may be used. The paramagnetic and superparamagnetic agents may also be coadministered separately, if desired.

Brief Summary Text (416):
The stabilizing materials and/or vesicles of the present invention, and especially the vesicles, may serve not only as effective carriers of the superparamagnetic agents described above, but also may improve the effect of the susceptibility contrast agents. Superparamagnetic contrast agents include metal oxides, particularly iron oxides but including manganese oxides, and as iron oxides, containing varying amounts of manganese, cobalt and nickel which experience a magnetic domain. These agents are nano or microparticles and have very high bulk susceptibilities and transverse relaxation rates. The larger particles, for example, particles having diameters of about 100 nm, have much higher R.sub.2 relaxivities as compared to R1 relaxivities. The smaller particles, for example, particles having diameters of about 10 to about 15 nm, have somewhat lower R2 relaxivities, but much more balanced R1 and R2 values. Much smaller particles, for example, monocrystalline iron oxide particles having diameters of about 3 to about 5 nm, have lower R2 relaxivities, but probably the most balanced R1 and R2 relaxation rates. Ferritin can also be formulated to encapsulate a core of very high relaxation rate superparamagnetic iron. It has been discovered that the lipid and/or vesicle compositions, especially vesicle compositions, including gas filled vesicles, can increase the efficacy and safety of these conventional iron oxide based MRI contrast agents.

Brief Summary Text (418):
Without being bound to any particular theory or theories of operation, it is believed that the vesicles of the present invention increase the efficacy of the superparamagnetic contrast agents by several mechanisms. First, it is believed that the vesicles function to increase the apparent magnetic concentration of the iron

oxide particles. Also, it is believed that the vesicles increase the apparent rotational correlation time of the MRI contrast agents, including paramagnetic and superparamagnetic agents, so that relaxation rates are increased. In addition, the vesicles appear to increase the apparent magnetic domain of the contrast medium according to the manner described hereinafter.

Brief Summary Text (419):
Certain of the vesicles of the present invention, and especially vesicles formulated from lipids, may be visualized as flexible spherical domains of differing susceptibility from the suspending medium, including, for example, the aqueous suspension of the contrast medium or blood or other body fluids, for example, in the case of intravascular injection or injection into other body locations. In the case of ferrites or iron oxide particles, it should be noted that the contrast provided by these agents is dependent on particle size. This phenomenon is very common and is often referred to as the "secular" relaxation of the water molecules. Described in more physical terms, this relaxation mechanism is dependent upon the effective size of the molecular complex in which a paramagnetic atom, or paramagnetic molecule, or molecules, may reside. One physical explanation may be described in the following Solomon-Bloembergen equations which define the paramagnetic contributions as a function of the T.sub.1 and T.sub.2 relaxation times of a spin 1/2 nucleus with gyromagnetic ratio g perturbed by a paramagnetic ion:

Brief Summary Text (422):
A few large particles may have a much greater effect than a larger number of much smaller particles, primarily due to a larger correlation time. If one were to make the iron oxide particles very large however, increased toxicity may result, and the lungs may be embolized or the complement cascade system may be activated. Furthermore, it is believed that the total size of the particle is not as important as the diameter of the particle at its edge or outer surface. The domain of magnetization or susceptibility effect falls off exponentially from the surface of the particle. Generally speaking, in the case of dipolar (through space) relaxation mechanisms, this exponential fall off exhibits an r.sup.6 dependence for a paramagnetic dipole-dipole interaction. Interpreted literally, a water molecule that is 4 angstroms away from a paramagnetic surface will be influenced 64 times less than a water molecule that is 2 angstroms away from the same paramagnetic surface. The ideal situation in terms of maximizing the contrast effect would be to make the iron oxide particles hollow, flexible and as large as possible. It has not been possible to achieve this heretofore and it is believed that the benefits have been unrecognized heretofore also. By coating the inner or outer surfaces of the vesicles with the contrast agents, even though the individual contrast agents, for example, iron oxide nanoparticles or paramagnetic ions, are relatively small structures, the effectiveness of the contrast agents may be greatly enhanced. In so doing, the contrast agents may function as an effectively much larger sphere wherein the effective domain of magnetization is determined by the diameter of the vesicle and is maximal at the surface of the vesicle. These agents afford the advantage of flexibility, namely, compliance. While rigid vesicles might lodge in the lungs or other organs and cause toxic reactions, these flexible vesicles slide through the capillaries much more easily.

Brief Summary Text (423):
In contrast to the flexible vesicles described above, it may be desirable, in certain circumstances, to formulate vesicles from substantially impermeable polymeric materials including, for example, polymethyl methacrylate. This would generally result in the formation of vesicles which may be substantially impermeable and relatively inelastic and brittle. In embodiments involving diagnostic imaging, for example, ultrasound, contrast media which comprise such brittle vesicles would generally not provide the desirable reflectivity that the flexible vesicles may provide. However, by increasing the power output on ultrasound, the brittle microspheres can be made to rupture, thereby causing acoustic emissions which can be detected by an ultrasound transducer.

Other Reference Publication (3):
Abstract of Nomura et al., "US Contrast Enhancement of Hepatic Tumor with Helium Gas Microbubbles: A Preliminary Report", Jpn. J. Med. Ultrasonics, 1991, 18(5), (Japanese with English language abstract).

Other Reference Publication (6):
Hynynen et al., "The Usefulness of a Contrast Agent and Gradient Recalled Acquisition in a Steady-State Imaging Sequence for Magnetic Resonance Imaging-Guided Noninvasive Ultrasound Surgery", Investigative Radiology, 1994, 29(10), 897-903.

Other Reference Publication (14):
Cheng, et al., "The Production and Evaluation of Contrast-Carrying Liposomes Made with an Automatic High Pressure System", Investigative Radiology, vol. 22, pp. 47-55 (1987).

Other Reference Publication (19):
Mattrey et al., "Perfluorochemicals as US Contrast Agents for Tumor-Imaging and Hepatosplenography: Preliminary Clinical Results", Radiology, vol. 163, pp. 339-343 (1987).

Other Reference Publication (20):
Mattrey et al., "Perfluoroctylbromide: A Liver/Spleen-Specific and Tumor Imaging Ultrasound Contrast Material", Radiology, vol. 145, pp. 759-762 (1982).

Other Reference Publication (21):
Keller et al., "Successful Left Ventricular Opacification Following Peripheral Venous Injection of Sonicated Contrast Agent: An Experimental Evaluation", LV Contrast Echocardiography, vol. 114, No. 3, pp. 570-575 (1987).

Other Reference Publication (22):
Feinstein et al., "Two-Dimensional Contrast Echocardiography, I: In Vitro Development and Quantitative Analysis of Echo Contrast Agents", JACC, vol. 3, No. 1, pp. 14-20 (1984).

Other Reference Publication (23):
Ten Cate et al., "Two-Dimensional Contrast Echocardiography, II: Transpulmonary Studies", JACC, vol. 3, No. 1, pp. 21-27 (1984).

Other Reference Publication (34):
Violante et al., "Particulate Suspensions as Ultrasonic Contrast Agents for Liver and Spleen", Inv. Rad., vol. 23, pp. S294-S297, Sep. 1988.

Other Reference Publication (35):
Fritzsch et al., "Preclinical and Clinical Results with and Ultrasonic Contrast Agent", Inv. Rad., vol. 23, pp. S302-S305, Sep. 1988.

Other Reference Publication (77):
Zhou et al., "Targeted delivery of DNA by liposomes and polymers", J. of Controlled Release 1992, 19:269-274.

Other Reference Publication (85):
Moseley, et al., Microbubbles: A Novel MR Susceptibility Contrast Agent, abstract, 1991 Napa, California Meeting of the Society for Magnetic Resonance in Medicine.

Other Reference Publication (89):
Abstract of Dittrich, "Cardiac Muscle Ischemia and Infarction", The Second Annual International Symposium on Contrast Agents in Diagnostic Ultrasound, Atlantic City, NJ (May 7, 1996).

Other Reference Publication (90):
Abstract of Pantely, "Intravenous Contrast Echocardiography-Tissue Imaging & Quantification of Coronary Blood Flow", The Second Annual International Symposium on Contrast Agents in Diagnostic Ultrasound, Atlantic City, NJ (May 7, 1996).

Other Reference Publication (91):
Schutt et al., "Osmotically Stabilized Microbubble Sonographic Contrast Agents", Acad. Radiol., vol. 3, Suppl. 2, pp. S188-S190 (Aug. 1996).

Other Reference Publication (98):

Mattrey et al., Gas Emulsions as Ultrasound Contrast Agents; Preliminary Results in Rabbits and Dogs, Investigative Radiology, vol. 29, Jun. Supp. 2, pp. S139-S141, 1994.

Other Reference Publication (99):
Meltzer et al., Transmission of Ultrasonic Contrast Through the Lungs, Ultrasound in Med. & Biol., vol. 7, No. 4, 377-384, 1981.

Other Reference Publication (102):
Ophir et al., "Contrast Agents in Diagnostic Ultrasound", Ultrasound in Med. & Biol., vol. 15, No. 4, pp. 319-333 (1989).

Other Reference Publication (108):
Unger et al., "Liposomal MR Contrast Agents", J. Liposome Research, 1994, 4(2), 811-834.

Other Reference Publication (109):
Feinstein, Steven B., "Myocardial Perfusion Imaging: Contrast Echocardiography Today and Tomorrow," Journal of the American College of Cardiology, 8(1) :251-253 (1986).

Other Reference Publication (110):
Keller et al., "The Behavior of Sonicated Albumin Microbubbles Within the Microcirulation: A Basis for Their Use During Myocardial Contrast Echocardiography", Circulation Res., 65(2) :458-465 (1989).

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Barnhart et al., "Characteristics of Albunex.TM.: Air-Filled Microspheres for Echocardiography Contrast Enhancement," Investigative Radiology, 25:S162-164 (1990).

Other Reference Publication (121):
Wheatley et al., "Contrast Agents for Diagnostic Ultrasound: Development and Evaluation of Polymer-Coated Microbubbles," Biomaterials, 11:713-717 (1990).

Other Reference Publication (150):
Villanueva et al., "Characterization of Spatial Patters of Flow Within the Reperfused Myocardium by Myocardial Contrast Echocardiography", Circulation, vol. 88, No. 6, pp. 2596-2606 (Dec. 1993).

Other Reference Publication (166):
Ulendorf, "Physics of Ultrasound Contrast Imaging: Scattering in the Linear Range", IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control, 1994, 41(1), 70-79.

Other Reference Publication (182):
Lindner et al., "Myocardial Perfusion Characteristics and Hemodynamic Profile of MRX-115, a Venous Echocardiographic Contrast Agent, During Acute Myocardial Infarction", J. Am. Soc. Echocardiography, 1998, 11(1), 36-46.

Other Reference Publication (185):
Desir et al., "Assessment of regional myocardial perfusion with myocardial contrast echocardiography in a canine model of varying degrees of coronary stenosis", Am. Heart J., Jan., 1994, 127(1), 56-63.

L7: Entry 317 of 424 File: USPT

DOCUMENT-IDENTIFIER: US 6001395 A
TITLE: Polymeric lamellar substrate particles for drug delivery

Brief Summary Text (5):
Many purified, synthetic or inactivated antigens such as Tetanus toxoid are poorly immunogenic and usually require several parenteral doses to confer adequate protection. Adsorption of vaccine antigens onto adjuvants such as Alum is a common method for enhancing the immunogenicity. A wide variety of substances, both biological and synthetic, have been used as adjuvants including mycobacteria, oil emulsions, liposomes, polymer microparticles and mineral gels. A range of 24 different adjuvants was recently investigated by Stieneker et al (1995) for inactivated HIV virus encompassing many of the adjuvant systems currently under investigation. However, only Aluminium hydroxide "Alum" has been approved for administration in humans but its use is often associated with adverse reactions.

Detailed Description Text (34):
Surface modifying polymers include the block copolymers based on polyethylene oxide and polypropylene oxide (POLOXAMERS.TM., POLOXAMINES.TM.) and tetra-functional block copolymers derived from the sequential addition of propylene oxide and ethylene oxide to ethylene diamine (POLOXAMINES.TM.), polyvinylalcohol, polyvinylpyrrolidone, sorbitan esters such as sorbitan monostearate (SPAN 60.TM.), polysorbates (TWEEN.TM.), polyoxyethylene fatty esters, phospholipids such as lecithin, lysophosphatidylcholine (LPC), fatty acids, stearic acid, stearates and their derivatives with for example, polyoxyethylene.

Detailed Description Text (40):
Examples of polysaccharides useful as surface modifiers include dextran, xanthan, chitosan, chitosan lactate, chitosan glutamate, pectin, dextrin, maltodextrin, hyaluronic acid, cellulose, starch, hydroxyethyl starch, pullulan, inulin, alginates, heparin and heparin-like synthetic polymers and their respective derivatives. Conjugates of PEG and polysaccharides for example have been described by Duval et al in Carbohydrate Polymers, 15 233-242 (1991).

Detailed Description Text (58):
It has been found that by using crystallisable polymers, the above precipitation method will form lamellar particles, in contrast to the prior art spherical particles formed using amorphous polymers.

L7: Entry 320 of 424 File: USPT

DOCUMENT-IDENTIFIER: US 5997898 A
TITLE: Stabilized compositions of fluorinated amphiphiles for methods of therapeutic delivery

Brief Summary Text (2):
The present invention relates to novel compositions for ultrasound. More particularly, the present invention relates to novel compositions of fluorinated amphiphilic compounds for use as contrast agents for ultrasound.

Brief Summary Text (6):
Ultrasound imaging techniques typically involve the use of contrast agents. Contrast agents are used to improve the quality and usefulness of images which are obtained via ultrasound. Exemplary contrast agents include, for example, suspensions of solid particles, emulsified liquid droplets, and gas-filled bubbles. See, e.g., Hilmann et al., U.S. Pat. No. 4,466,442, and published International Patent Applications WO 92/17212 and WO 92/21382.

Brief Summary Text (7):
The quality of images produced from ultrasound has improved significantly. Nevertheless, further improvement is needed, particularly with respect to images involving vasculature in tissues that are perfused with a vascular blood supply. Accordingly, there is a need for improved ultrasound techniques, including improved contrast agents, which are capable of providing medically useful images of the vasculature and vascular-related organs.

Brief Summary Text (8):
The reflection of sound from a liquid-gas interface is extremely efficient. Accordingly, bubbles, including gas-filled bubbles, are useful as contrast agents. The term "bubbles", as used herein, refers to vesicles which are generally characterized by the presence of one or more membranes or walls surrounding an internal void that is filled with a gas or a precursor thereto. Exemplary bubbles include, for example, liposomes, micelles and the like. As discussed more fully hereinafter, the effectiveness of bubbles as contrast agents depends upon various factors, including, for example, the size, elasticity and/or stability of the bubble.

Brief Summary Text (10):
However, bubble size is limited by the diameter of capillaries through which the bubbles must pass. Contrast agents which comprise bubbles having a diameter of greater than 10 .mu.m are generally dangerous since microvessels may be occluded. Accordingly, it is desired that greater than about 99% of the bubbles in a contrast agent have a diameter of less than 10 .mu.m. Mean bubble diameter is important also, and should be greater than 1 .mu.m, with greater than 2 .mu.m being preferred. The volume weighted mean diameter of the bubbles should be about 7 to 10 .mu.m.

Brief Summary Text (12):
The effectiveness of a contrast agent involving bubbles is also dependent on the bubble concentration. Generally, the higher the bubble concentration, the greater the reflectivity of the contrast agent.

Brief Summary Text (13):
Another important characteristic which is related to the effectiveness of bubbles as

contrast agents is bubble stability. As used herein, particularly with reference to gas-filled bubbles, "bubble stability" refers to the ability of bubbles to retain gas entrapped therein after exposure to a pressure greater than atmospheric pressure. To be effective as contrast agents, bubbles generally need to retain an amount of the entrapped gas in vivo. It is also highly desirable that, after release of the pressure, the bubbles return to their original size. This is referred to generally as "bubble resilience."

Brief Summary Text (14):
Bubbles which lack desirable stability provide poor contrast agents. If, for example, bubbles release the gas entrapped therein in vivo, reflectivity is diminished. Similarly, the size of bubbles which possess poor resilience will be decreased in vivo, also resulting in diminished reflectivity.

Brief Summary Text (15):
The stability of bubbles disclosed in the prior art is generally inadequate for use as contrast agents. For example, the prior art discloses bubbles, including gas-filled liposomes, which comprise lipoidal walls or membranes. See, e.g., Ryan et al., U.S. Pat. Nos. 4,900,540 and 4,544,545; Tickner et al., U.S. Pat. No. 4,276,885; Klaveness et al., WO 93/13809 and Schneider et al., EPO 0 554 213 and WO 91/15244. The stability of the bubbles disclosed in these references is poor in that as the solutions in which the bubbles are suspended become diluted, for example, in vivo, the walls or membranes of the bubbles are thinned. This results in a greater likelihood of rupture of the bubbles.

Brief Summary Text (17):
Prior art techniques for stabilizing bubbles, including the use of crosslinked materials, suffer from various drawbacks. For example, the crosslinked materials described, for example, in Giddey et al., U.S. Pat. No. 5,310,540 and Klaveness et al., WO 92/17212, lack biocompatibility or possess unknown metabolic fates. Added costs are also incurred with the use of additional materials and process steps necessary for crosslinking. In addition, crosslinking can impart rigidity to the membranes or walls of the bubbles. This results in bubbles having reduced elasticity and, therefore, a decreased ability to deform and pass through capillaries. Thus, there is a greater likelihood of occlusion of vessels with prior art contrast agents that are stabilized via crosslinking.

Brief Summary Text (18):
Accordingly, new and/or better stabilized contrast agents and methods for providing same are needed. The present invention is directed to this, as well as other, important ends.

Brief Summary Text (36):
Still another aspect of the invention relates to a method of providing an image of an internal region of a patient. The method comprises administering to the patient a contrast medium comprising a stabilized composition comprising a gas and a fluorinated amphiphilic compound. The method further comprises scanning the patient using ultrasound to obtain visible images of the region.

Brief Summary Text (38):
Yet another aspect of the invention relates to a method for diagnosing the presence of diseased tissue in a patient. The method involves administering to the patient a contrast medium comprising a stabilized composition comprising a gas and a fluorinated amphiphilic compound. The method further involves scanning the patient using ultrasound to obtain visible images of any diseased tissue in the patient.

Brief Summary Text (58):
"Diagnostic agent" refers to any agent which is used in connection with methods for diagnosing the presence or absence of a disease in a patient. Exemplary diagnostic agents include, for example, contrast agents for use in connection with ultrasound, magnetic resonance imaging or computed tomography of a patient.

Brief Summary Text (65):
The present invention is directed, in part, to stabilized compositions which are useful, for example, as contrast agents for diagnostic and/or therapeutic

ultrasound. The compositions comprise, in combination with a gas and preferably in an aqueous carrier, a fluorinated amphiphilic compound. The fluorinated amphiphilic compounds, which are described in detail below, impart highly desirable properties to the compositions of the present invention. For example, it has been surprisingly and unexpectedly found that the fluorinated amphiphilic compounds are capable of stabilizing the present compositions, including preferred compositions which comprise vesicles. It has been found also that the present fluorinated amphiphilic compounds are capable of promoting the formation of vesicles, as well as improving the stability of the formed vesicles. In embodiments in which the vesicles comprise gas-filled and/or gaseous precursor-filled vesicles, the fluorinated amphiphilic compounds enable the vesicles to substantially retain the gas and/or gaseous precursor with minimal loss or leakage. This is surprising and unexpected and generally renders unnecessary the use of additional stabilizing materials, including, for example, surfactants, and stabilizing techniques, including, for example, crosslinking of the materials in the walls of the vesicles. As discussed above, such techniques are generally necessary in connection with contrast agents of the prior art. Moreover, the present fluorinated amphiphilic compounds are generally biocompatible and can be obtained with minimal effort and at minimal expense. Accordingly, the present invention is directed to simple and efficient methods for providing stabilized compositions, including vesicular compositions, for use as ultrasound contrast agents.

Brief Summary Text (67):
In preferred embodiments, the fluorinated amphiphilic compounds are based on amphiphilic compounds, including lipids, and especially phospholipids, which comprise a polar head group including, for example, a phosphorylated head group, such as a phosphatidylcholine group, or a sulfated head group, and at least one nonpolar aliphatic chain, such as a palmitoyl group. In such embodiments, the fluorine atoms are preferably substituted on the nonpolar aliphatic chain portions of the involved amphiphilic compounds. As noted above, among the preferred amphiphilic compounds are phosphorylated and/or sulfated lipid compounds. It is contemplated that the term "phosphorylate", as used herein, encompasses phosphate groups with various valences, including, for example, PO.sub.3 and PO.sub.4 groups. Similarly, it is contemplated that the term "sulfated", as used herein, encompasses sulfate groups with various valences, including, for example, SO.sub.3 and SO.sub.4. It is contemplated that in these preferred lipid compounds, the phosphate group and/or the sulfate group are preferably located within the backbone portions of the lipid compounds. Thus, generally speaking, the phosphate and/or sulfate groups in the preferred phosphorylated and/or sulfated lipid compounds are desirably spaced from the end-portions of the compounds with, for example, alkyl groups, and as such, are referred to herein as "internal phosphate (and/or sulfate) groups". These preferred fluorinated amphiphilic compounds can be contrasted with prior art fluorinated compounds, including, for example, the class of compounds which are commercially available as ZONYL.TM. fluorosurfactants (DuPont Chemical Corp., Wilmington Del.), including the ZONYL.TM. phosphate salts and the ZONYL.TM. sulfate salts, which have terminal phosphate or sulfate groups. Representatives of these salts are disclosed, for example, in U.S. Pat. No. 5,276,146, wherein the ZONYL.TM. phosphate salt has the formula [F(CF.sub.2 CF.sub.2).sub.3-8 CH.sub.2 CH.sub.2 O].sub.1,2 P(O)(O.sup.- NH.sub.4.sup.+).sub.2,1 and the ZONYL.TM. sulfate salt has the formula F(CF.sub.2 CF.sub.2).sub.3-8 CH.sub.2 CH.sub.2 SCH.sub.2 CH.sub.2 N.sup.+ (CH.sub.3).sub.3.sup.-OSO.sub.2 OCH.sub.3. In contrast to the preferred phosphorylated and sulfated lipid compounds involved in the present invention, the ZONYL.TM. phosphate and sulfate salts, as depicted above, include phosphate and sulfate moieties in the terminal portions of the disclosed compounds.

Brief Summary Text (115):
The stabilized compositions of the present invention also comprise a gas, and preferably, an inert gas. The gases provide the compositions with enhanced reflectivity, particularly in vesicular composition in which the gas is entrapped within the vesicles. This increases their effectiveness as contrast agents.

Brief Summary Text (117):
It is contemplated that mixtures of different types of gases, including mixtures of a perfluorocarbon gas and another type of gas, such as air, can also be used in the compositions of the present invention. The gases discussed in Quay, International

Application WO 93/05819, including the high "Q" factor gases described therein, may be used also. The disclosures of Quay, International Application WO 93/05819 are incorporated herein by reference in their entirety. In addition, paramagnetic gases and gases of isotopes, such as .sup.17 O, may be used. Other gases, including the gases exemplified above, would be readily apparent to one skilled in the art based on the present disclosure. The gases can be selected, as desired, to provide compositions which are suitable for use as contrast agents in ultrasound, as well as other diagnostic techniques, such as computed tomography (CT) and magnetic resonance imaging (MRI).

Brief Summary Text (138):
In certain preferred embodiments, the additional amphiphilic materials comprise a lipid compound. Suitable lipids include, for example, phospholipids, such as phosphatidylcholine with both saturated and unsaturated fatty acids, including dioleoylphosphatidylcholine, dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine; phosphatidylethanolamines, such as dipalmitoylphosphatidylethanolamine, dioleoylphosphatidylethanolamine, N-succinyldioleoylphosphatidylethanolamine and 1-hexadecyl-2-palmitoylglycerophosphoethanolamine; phosphatidylserine; phosphatidylglycerol; sphingolipids; glycolipids, such as ganglioside GM1; glucolipids; sulfatides; glycosphingolipids; phosphatidic acids, such as dipalmitolylphosphatidic acid; palmitic acid; stearic acid; arachidonic acid; oleic acid; lipids bearing polymers, such as polyethyleneglycol or polyvinylpyrrolidone; cholesterol and cholesterol hemisuccinate; 12-(((7'-diethylaminocoumarin-3-yl)carbonyl)methylamino)octadecanoic acid; N-[12-(((7'-diethylaminocoumarin-3-yl)carbonyl)methylamino)octadecanoyl]-2 -aminopalmitic acid; cholesteryl-(4'-trimethylamino)butanoate; 1,2-dioleoyl-sn-glycerol; 1,2-dipalmitoyl-sn-3-succinylglycerol; 1,3-dipalmitoyl-2-succinylglycerol; and palmitoylhomocysteine.

Brief Summary Text (142):
The present compositions can also comprise, if desired, one or more neutral or positively or negatively charged materials. Exemplary neutral materials include, for example, oils, such as peanut oil, canola oil, olive oil, safflower oil and corn oil; lecithin; sphingomyelin; cholesterol and derivatives thereof; squalene; terpenes and terpenoid compounds; triglycerides; gums, such as xanthan, tragacanth, locust bean, guar and carrageenan gums; methoxylated pectin; starch; agarose; cellulose and semi-synthetic cellulose, for example, methyl cellulose, hydroxyethyl cellulose, methoxy cellulose and hydroxypropyl cellulose; nonionic materials, including, for example, polyoxyethylene fatty acid esters, polyoxyethylene fatty alcohols, polyoxyethylene fatty alcohol ethers, polyoxyethylated sorbitan fatty acid esters, glycerol polyethylene glycol oxystearate, glycerol polyethylene glycol ricinoleate, ethoxylated soybean sterols, ethoxylated castor oil, polyoxyethylenepolyoxypropylene polymers and polyoxyethylene fatty acid stearates; acacia; agar; bentonites, including purified bentonite; magma; carbomer 934P; dextrin; gelatin; di- and trihydroxy substituted alkanes and their polymers, including polyvinylalcohol; mono-, di- and triglycerides; amino alcohols; monosaccharides or sugar alcohols, such as erythrose, threose, ribose, arabinose, xylose, lyxose, fructose, sorbitol, mannitol and sedoheptulose, with preferred monosaccharides being fructose, mannose, xylose, arabinose, mannitol and sorbitol; and disaccharides, such as lactose, sucrose, maltose and cellobiose.

Brief Summary Text (161):
As those skilled in the art will recognize, any of the present stabilized compositions and/or formulations may be lyophilized for storage, and reconstituted, for example, with an aqueous medium (such as sterile water or phosphate buffered solution, or aqueous saline solution), with the aid of vigorous agitation. To prevent agglutination or fusion of the fluorinated amphiphilic compounds and/or additional amphiphilic compounds as a result of lyophilization, it may be useful to include additives which prevent such fusion or agglutination from occurring. Additives which may be useful include sorbitol, mannitol, sodium chloride, glucose, trehalose, polyvinylpyrrolidone and poly(ethylene glycol), for example, PEG 400. These and other additives are described in the literature, such as in the U.S. Pharmacopeia, USP XXII, NF XVII, The United States Pharmacopeia, The National Formulary, United States Pharmacopeial Convention Inc., 12601 Twinbrook Parkway,

Rockville, Md. 20852, the disclosures of which are hereby incorporated herein by reference in their entirety. Lyophilized preparations generally have the advantage of greater shelf life.

Brief Summary Text (168):
Ultrasonic imaging techniques, including second harmonic imaging, are well known in the art, and are described, for example, in Uhlendorf, "Physics of Ultrasound Contrast Imaging: Scattering in the Linear Range", IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control, Vol. 14(1), pp. 70-79 (1994) and Sutherland, et al., "Color Doppler Myocardial Imaging: A New Technique for the Assessment of Myocardial Function", Journal of the American Society of Echocardiography, Vol. 7(5), pp. 441-458 (1994), the disclosures of which are hereby incorporated herein by reference in their entirety.

Brief Summary Text (172):
Fluorinated amphiphilic formulations can be formulated to be sufficiently stable in the vasculature such that they circulate throughout the body and provide blood pool equilibration. As one skilled in the art would recognize, based on the present disclosure, the formulations, including those which comprise suspensions, emulsions and/or vesicles, such as liposomes and micelles, may be coated with certain materials to minimize uptake by the reticuloendothelial system. Suitable coatings include, for example, gangliosides and glycolipids which bind saccharide moieties, such as glucuronate, galacturonate, guluronate, poly(ethylene glycol), poly(propylene glycol), polyvinylpyrrolidone, poly(vinyl alcohol), dextran, starch, phosphorylated and sulfonated mono-, di-, tri-, oligo- and polysaccharides and albumin. Provided that the circulation half-life of the formulations is of a sufficient period of time, they will generally pass through the target tissue while passing through the body. In the case of formulations which comprise a bioactive agent, energy, for example, sonic energy, may be focused on the tissue to be treated, for example, diseased tissue. The bioactive agent will then be released locally in the target tissue. The inventors have found also that antibodies, carbohydrates, peptides, glycopeptides, glycolipids and lectins also assist in the targeting of tissue. Accordingly, these materials may be incorporated into the fluorinated amphiphilic formulations also.

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Other Reference Publication (38):
Cheng et al., "The Production and Evaluation of Contrast-Carrying Liposomes Made with an Automatic High Pressure System", Investigative Radiology, vol. 22, pp. 47-55 (1987).

Other Reference Publication (43):
Mattrey et al., "Perfluorochemicals as US Contrast Agents for Tumor-Imaging and Hepatosplenography: Preliminary Clinical Results", Radiology, vol. 163, pp. 339-343 (1987).

Other Reference Publication (44):
Mattrey et al., "Perfluoroctylbromide: A Liver/Spleen-Specific and Tumor Imaging Ultrasound Contrast Material", Radiology, vol. 145, pp. 759-762 (1982).

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Keller et al., "Successful Left Ventricular Opacification Following Peripheral Venous Injection of Sonicated Contrast Agent: An Experimental Evaluation", LV Contrast Echocardiography, vol. 114, No. 3, pp. 570-575 (1987).

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Ten Cate et al., "Two-Dimensional Contrast Echocar-diography, II: Transpulmonary Studies", JACC, vol. 3, No. 1, pp. 21-27 (1984).

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